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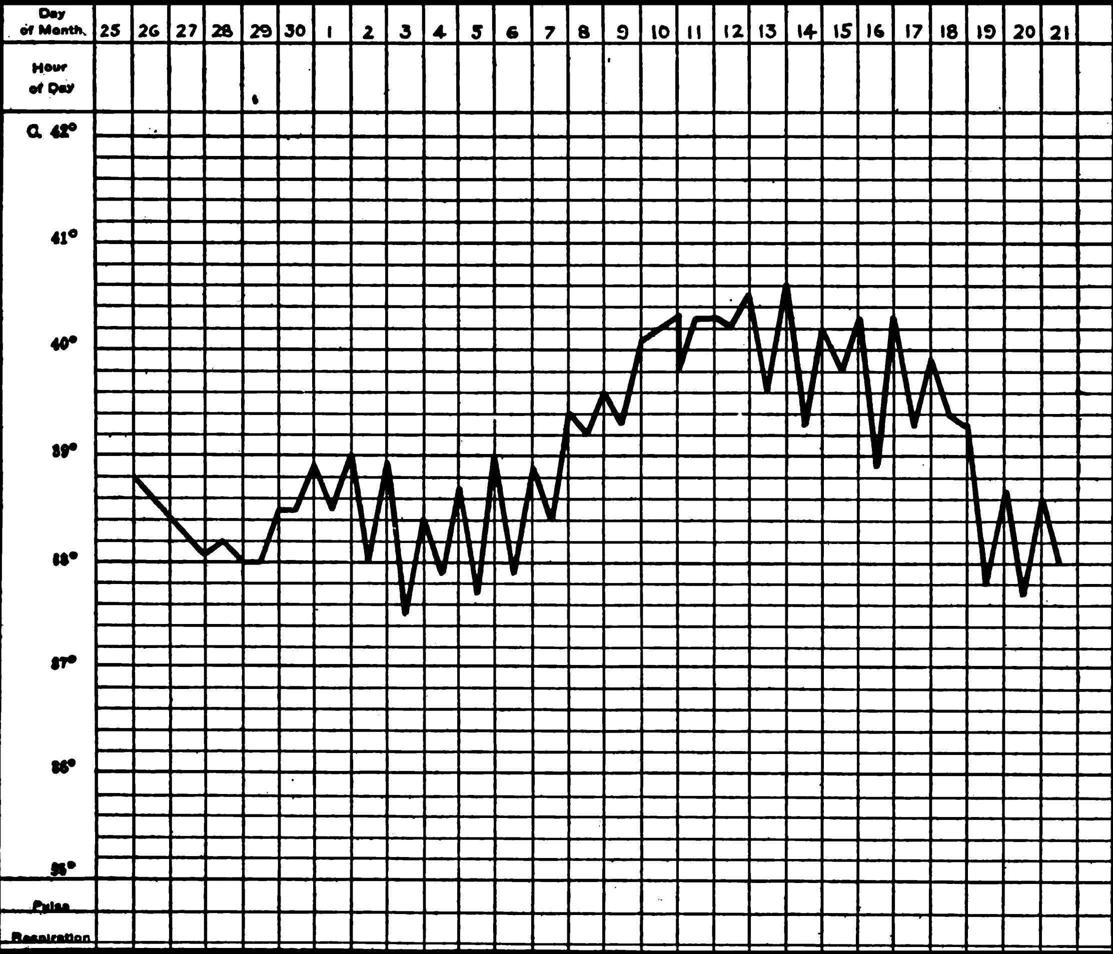

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TREASURY DEPARTMENT
United States Public Health Service

HYGIENIC LABORATORY—BULLETIN No. 86
October, 1912

# COLLECTED STUDIES ON TYPHUS.

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# PREFACE.

There are brought together in this bulletin the series of papers on experimental typhus that have recently (1911–12) appeared in the Public Health Reports, together with one paper now published for the first time. For the sake of completeness and because they are now out of print it was considered desirable to include the series of papers that appeared in the Public Health Report during the winter of 1909–10.

# 1.—ON THE RELATION OF ROCKY MOUNTAIN SPOTTED FEVER TO THE TYPHUS FEVER OF MEXICO.—A PRELIMINARY NOTE.[1]

By John F. Anderson, *Director Hygienic Laboratory*, and Joseph Goldberger, *Passed Assistant Surgeon, United States Public Health Service.*

Several observers and writers have called attention to the close clinical resemblance of Rocky Mountain spotted fever to typhus. One of us having seen typhus fever in England, and later the spotted fever in Montana, was also struck by the clinical similarity of the two diseases.

Typhus fever being one of the quarantinable diseases under the regulations of the Treasury Department, it seemed desirable both on theoretical and practical grounds to take up the study of typhus fever in Mexico in order to determine whether the two diseases are really identical, particularly as the work of Ricketts had developed a basis for the identification of the Rocky Mountain disease. Ricketts has shown that Rocky Mountain spotted fever has a definite clinical course in certain animals, particularly guinea pigs and monkeys, in which it also produces characteristic anatomical lesions. He has shown that in the guinea pig, after intraperitoneal inoculation of blood of spotted fever, there appears in from two to five days a distinct rise in the temperature of the animal. The fever continues to rise for two or three days, after which it maintains itself at about the level which it has attained for two or three days longer. At the end of this time the animal either dies or the fever begins to subside. The objective signs of the infection are a roseolar eruption, with hemorrhage into and frequently gangrene of the external genitals. At necropsy the most striking features are a greatly enlarged spleen and swollen and hemorrhagic lymph nodes.

With these facts in mind, we took up the study of typhus as it presents itself in Mexico City. Our observations of the disease in the Hospital General bring out certain definite clinical differences between this disease and the Rocky Mountain fever. These differences we will discuss in a later paper.

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[1] Reprinted from the Public Health Reports, vol. 24, No. 50, Dec. 10, 1909.

Experimentally, in guinea pigs, the intraperitoneal injection of blood drawn from patients between the eighth and fifteenth day after the onset of typhus fever has failed to produce either the signs or symptoms above briefly described for Rocky Mountain spotted fever. We have made similar inoculations into monkeys, rabbits, and rats, the results of which we are not as yet in a position to present. In our opinion, however, the negative results in the guinea pig are conclusive evidence of the nonidentity of the two diseases.

For the laboratory and clinical facilities which we have enjoyed we are greatly indebted to many of our Mexican friends and colleagues, amongst whom, even in this brief note, we can not forbear to mention Drs. Eduardo Liceága, Octaviano Gonzalez-Fabella, Fernando Lopez, and Genaro Escalono.

# 2.—A NOTE ON THE ETIOLOGY OF "TABARDILLO," THE TYPHUS FEVER OF MEXICO.[1]

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By JOHN F. ANDERSON, *Director Hygienic Laboratory*, and JOSEPH GOLDBERGER, *Passed Assistant Surgeon, United States Public Health Service.*

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In a previous note[2] on the basis of the results of guinea-pig inoculations, we have reported on the nonidentity of Rocky Mountain spotted fever and the typhus fever of Mexico, and at the same time stated that we had made inoculations with blood of typhus fever into rabbits and monkeys, the results of which at that time we were not ready to present. Sufficient time has now elapsed since the inoculations in these animals were made to confirm the results of the experiments in the guinea pigs.

We have now to report some further results of the work, which, while primarily started for the purpose of ascertaining the relationship between Rocky Mountain spotted fever and Mexican typhus, have an important bearing on the problem of the etiology of the typhus fever of Mexico, locally known as "Tabardillo." Up to the date of the present writing (December 16) we have studied 12 cases of this disease. For purposes of easy reference we have numbered these cases serially, and in a later paper we hope to give a summary of the important clinical features presented by each.

Aside from the clinical aspects, these studies embrace cultures and animal inoculations.

## CULTURES.

Cultures were made with the blood from cases Nos. 1, 2, 3, 4, 5, 6, 7, 8, and 11. The blood was drawn from the general circulation. The cases furnishing this blood were in different stages of the disease—from the fifth to the fifteenth day. The blood was planted in standard bouillon in flasks containing 50 c. c., in fermentation tubes, freshly prepared, and on agar or Loeffler's serum slants.

In no instance did we obtain any visible growth.

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[1] Reprinted from the Public Health Reports, vol. 24, No. 52, Dec. 24, 1909.

[2] United States Public Health Reports, Dec. 10, 1909, vol. 24, p. 1861.

## MONKEYS.

We made blood inoculations in two monkeys:

"Bedalia," a female *rhesus*, was inoculated intraperitoneally November 27, 1909, with 10 c. c. of defibrinated blood from case No. 6, on November 30, 1909, with 5 c. c. from case No. 8, and on December 2, 1909, with 5 c. c. of defibrinated blood from case No. 11 diluted with an equal volume of physiological salt solution.

On December 7, 1909, 11 days from the first and 5 from the last inoculation, the temperature of the animal rose and continued to rise, reaching its fastigium on the third day and recording a temperature of 40.6° C. on the seventh day of illness.

On December 19, 1909, there was a critical drop in the temperature of the animal to normal.

After the first two days of fever the animal began to show marked symptoms of illness. On the fourth day blood was drawn from the heart and used for passage into another monkey.

"Jerry," a male capuchin, was inoculated intraperitoneally on November 28, 1909, with 4 c. c. of defibrinated blood from case No. 7, and on December 2, 1909, with 4 c. c. from case No. 11 diluted with an equal volume of physiological salt solution.

On December 3, 1909, six days after the first and one day after the second inoculation, the temperature of the animal began to rise, reaching its maximum, 40.5° C., on the fourth day of illness. This animal showed no definite symptoms other than a slight diminution in his appetite and a marked thirst. On December 11, 1909, his temperature had declined to 38.7° C. The following morning the temperature recorded was 36.6°. It continued to fall, interrupted by more or less marked evening exacerbations, until in the morning of December 16, 1909, the mercury barely rose to 34.3° C. The animal's temperature gradually returned to normal on December 18, 1909.

# 3.—ON THE INFECTIVITY OF TABARDILLO OR MEXICAN TYPHUS FOR MONKEYS AND STUDIES ON ITS MODE OF TRANSMISSION.[1]

By John F. Anderson, *Director Hygienic Laboratory*, and Joseph Goldberger,
*Passed Assistant Surgeon, United States Public Health Service.*

In two previous notes[2] we reported some results of work upon tabardillo, the typhus fever of Mexico. In the second of these notes we recorded the experimental production of fever after the inoculation with blood from cases of human tabardillo in two species of monkeys; one "Bedalia," a female *Macacus rhesus*, and the other "Jerry," a male *Cebus capuchinus*. In order to determine whether the fever thus produced was tabardillo, we decided to test the immunity of these two monkeys by again inoculating them with virulent blood.

On January 7, 26 days after the subsidence of the fever, "Jerry" was given intraperitoneally 8 cubic centimeters of defibrinated blood from case No. 19. This blood was drawn from the general circulation on the tenth day of the disease, and inoculations with it on ordinary culture media showed no visible growth. The accidental death of the control animal made it desirable to reinoculate this monkey. Accordingly, on January 11, 30 days from the subsidence of the fever, "Jerry" received 7 cubic centimeters of defibrinated blood from case No. 20. This blood was drawn from the general circulation on the eighth day of the disease and inoculations with it on ordinary culture media showed no visible growth.

On the same day 5 cubic centimeters of defibrinated blood from the same case (case No. 20) was injected intraperitoneally into "Bedalia," the female *rhesus*, whose attack of fever subsided critically 23 days before. The temperature of both monkeys was taken twice daily during a period of 14 days following the inoculations. No febrile reaction or other indication of illness was noted during this period, at the end of which we were obliged to terminate our observations.[1]

---

[1] Reprinted from the Public Health Reports, vol. 25, No. 7, Feb. 18, 1910.

[2] Anderson, John F., and Goldberger, Joseph: On the relation of Rocky Mountain spotted fever to the typhus fever of Mexico. A preliminary note. Public Health Reports, vol. 24, Dec. 10, 1909, p. 1861.

Same: A note on the etiology of "tabardillo," the typhus fever of Mexico. Public Health Reports, vol. 24, Dec. 24, 1909, p. 1941.

The succession of events after the inoculation of the two monkeys, " Bedalia " and " Jerry," particularly well defined in the former, with blood that gave no visible growth on ordinary culture media, namely, an incubation period of not less than 5 nor more than 11 days succeeded by a fever of 13 days whose onset, course, duration, and critical defervescence presented a most striking resemblance to the fever curve of this disease in man, as may be seen by comparing charts Nos. 1 and 2, left but little doubt in our minds that the fever produced in these animals although without a discernible eruption was due to the transfer of a living virus.

In order, however, to eliminate any possible doubt, it was decided first to reproduce the fever in two or more animals by the inoculation of virulent blood from a human case and then, if successful, to attempt to transfer the infection from those monkeys to a second series. Accordingly the following experiments were performed:

"Adela," a female *rhesus*, was inoculated intraperitoneally on January 11 with 6 cubic centimeters of defibrinated blood from case No. 20, this being a portion of the same blood as was used for testing the immunity of " Bedalia " and " Jerry." This blood, as has already been stated, was drawn from the general circulation on the eighth day of the disease and on ordinary culture media gave no visible growth. On January 19, eight days following the inoculation, the temperature of this animal began to rise, reaching a maximum of 40.6° C. on January 25.[2] Simultaneously with the rise in temperature the animal gave other indications of illness, such as diminution of the appetite, thirst, ruffled fur and attitude. On January 23, on the fifth day of illness, about 3 cubic centimeters of urine was obtained and found to contain a small percentage of albumin. The fever ended critically on January 30.

" Maria," a female *rhesus*, was inoculated intraperitoneally on January 11 with 8 cubic centimeters of defibrinated blood from case No. 20, this being another portion of the same blood as was used for the inoculation of "Adela," "Jerry," and "Bedalia." On January 19, eight days after the inoculation, the temperature of the animal began to rise, reaching 40.2° C. on the 24th. The following day the temperature was somewhat lower. After January 25 observation of this animal was suspended and not resumed until the afternoon of January 31, when the temperature was found to be normal and the animal in apparently good health. Beside the elevation of temperature this animal showed symptoms of illness similar to but not so marked as those presented by "Adela." This animal, though inoculated with a larger amount of blood than "Adela," presented the milder reaction. This seems to be worthy of note, inasmuch as Ricketts and Wilder[1] made a somewhat similar observation in their experiments.

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[1] On account of the illness of one of us (J. G.) it was necessary to terminate the work in Mexico City. Accordingly the experimental animals, with one exception ("Adela"), were shipped to the laboratory in Washington. For a period of five days, therefore, observations on them were necessarily suspended.

[2] On account of the gravity of her condition, this animal was left in Mexico on our departure for Washington. The temperatures recorded and other observations after January 25 were made and reported to us by Mr. Braulio B. Ramirez, technical assistant in the laboratory of the Superior board of health, to whom we wish here to express our obligations.

In view of the more marked reaction in the monkey "Adela" we selected this animal from which to draw blood for passage to a second series of monkeys. Accordingly, on January 23, four days after the onset of the fever in this monkey, blood was drawn from her heart and at once defibrinated. Eight and one-half cubic centimeters of the defibrinated blood was inoculated intraperitoneally into "Faustinella," a female rhesus.

On January 24, five days after the onset of fever, some more blood was aspirated from her heart and immediately defibrinated. Eight cubic centimeters of this defibrinated blood was inoculated intraperitoneally into "José," a male rhesus.

In broth and on blood-serum slants neither the defibrinated blood used for the inoculation of "Faustinella" nor that used for the inoculation of "José," gave any visible growth.

These animals were kept under observation up to and including January 25, after which date we were obliged to suspend observations.

Observations were resumed in the afternoon of January 31, at which time (eight days after inoculation) "Faustinella" showed a slight rise in temperature. This animal continued for several days with a somewhat elevated temperature, but gave no other indication of illness.

On resuming observations "José" was found (seven days after inoculation) with a temperature of 40.4° C. In the afternoon of February 2, the temperature reached 40.7° C. Two days later the temperature had dropped 2°. During the height of its fever this animal, with the exception of a slight diminution in appetite, showed but little other indication of sickness.

On February 4 blood was aspirated from the heart, immediately defibrinated, and 6 cubic centimeters injected intraperitoneally into another rhesus monkey. This blood was sterile upon ordinary culture media.

It is interesting to note that neither "Faustinella" nor "José" presented marked signs of illness during the period of observation.

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[1] Ricketts, H. T., and Wilder, Russell M.: The typhus fever of Mexico (tabardillo). Journ. Am. Med. Assn., vol. 54, Feb. 5, 1910, p. 463.

This may possibly be significant, in view of Nicolle's[1] experience indicating an attenuation of the virus by passage through the animals with which he experimented.

We have here then, first, a production of fever in two monkeys induced by a single intraperitoneal inoculation in each of defibrinated blood directly from a human case of tabardillo, after an incubation period of eight days in both animals, the blood used being sterile on ordinary culture media. The onset, course, duration, and defervescence of the fever, as observed in one of these animals ("Adela," chart No. 3) bears a close resemblance to the fever curve for "Bedalia" (chart No. 2) and that frequently observed in human cases (chart No. 1). Second, blood drawn from the heart of one of these monkeys ("Adela") four days after the onset of the fever, and again on the following day, although sterile on ordinary culture media, induced, when inoculated intraperitoneally into the monkeys "Faustinella" and "José," respectively, a fever resembling that of the animal from which the blood originated.

We believe, therefore, that this succession of events justifies the conclusion that in the inoculation of monkey from man, and in the subsequent passage from monkey to monkey, a living virus capable of multiplication was transferred.

## FILTRATION.

With a view of obtaining some idea of the size of the infecting agent we performed the following experiment: Six cubic centimeters of blood serum were obtained by centrifugalization of a portion of defibrinated blood from case No. 20, this being a portion of the same blood as was used for testing the immunity of "Bedalia" and "Jerry" and for the inoculation of "Adela" and "Maria." The blood serum was diluted with 12 cubic centimeters of physiological salt solution and then filtered through a Berkefeld filter. Fifteen cubic centimeters of the filtrate, representing approximately 5 cubic centimeters of the serum, was inoculated intraperitoneally into "Esther," a female *rhesus*, on January 11.

The temperature of this animal was taken twice daily up to and including January 25, a period of 14 days, when observations were temporarily suspended. On the afternoon of January 31, when observations were resumed, no febrile reaction or other manifestations of illness were noted. It would appear, therefore, that either the infecting organism is too large to pass through this filter or that it passed through in insufficient numbers to produce a reaction in this animal. Ricketts and Wilder[1] have reported that the virus failed to pass through a Berkefeld filter in an experiment made on the same date (January 11) as the one done by us.

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[1] Nicolle, Charles; Comte, C.; and Conseil, E.: Transmission expérimentale du typhus exanthematique par le pou du corps. C. R. des Acad. Sci., tome 149, No. 10, Sept. 6, 1909, p. 486.

It may be well to note here that portions of the blood serum from case No. 20 on January 11 were used, first, to test the immunity of "Bedalia" and "Jerry"; second, to inoculate "Adela" and "Maria"; and third, for the inoculation of "Esther," after filtration through a Berkefeld filter. As has already been recorded above, the monkeys "Bedalia" and "Jerry" showed their immunity by giving no reaction to the inoculation with this blood; "Adela" and "Maria" both became sick; "Esther," the animal inoculated with the filtered serum, showed no reaction.

## INSECT TRANSMISSION.

The peculiarities of the epidemiology of tabardillo must strike even the most casual reader who is familiar with the advances in our knowledge of the rôle of insects in disease transmission as highly suggestive of the existence of an intermediary host. We were very early convinced that this disease is not contagious, using this word in its ordinary sense. We could cite a long series of facts, both from the literature and from our own observations, in support of this view. We shall, however, content ourselves with citing the following:

F. J., adult, American, nonimmune. Lived at a hotel in Mexico City, but came in daily intimate contact with cases of tabardillo between November 22 and December 16, 1909. On the nights of January 5 and 6 he slept in a bed that had been occupied on January 2, 3, and 4 by a patient in the first three days of a well-marked attack of tabardillo (chart No. 1). None of the bedding or bedclothes had been in any way disturbed in the interval prior to their use by this individual. At the end of three days the bedclothes were changed, but with this exception the bed and room remained as they had been when occupied by the patient. F. J. inhabited this room for three weeks longer. On careful search no insects other than fleas were found in the room. During a period of observation of 17 days this man continued in his usual health.

The following data, for which we are indebted to Miss Ella Wilson, superintendent of the American Hospital in Mexico City, are significant in this connection. From January 9, 1908, to November 24, 1909, 30 cases of typhus fever had been treated in the typhus pavilion of that hospital. Each of these cases had had a special nurse who was an American nonimmune. None of these nurses contracted the disease.

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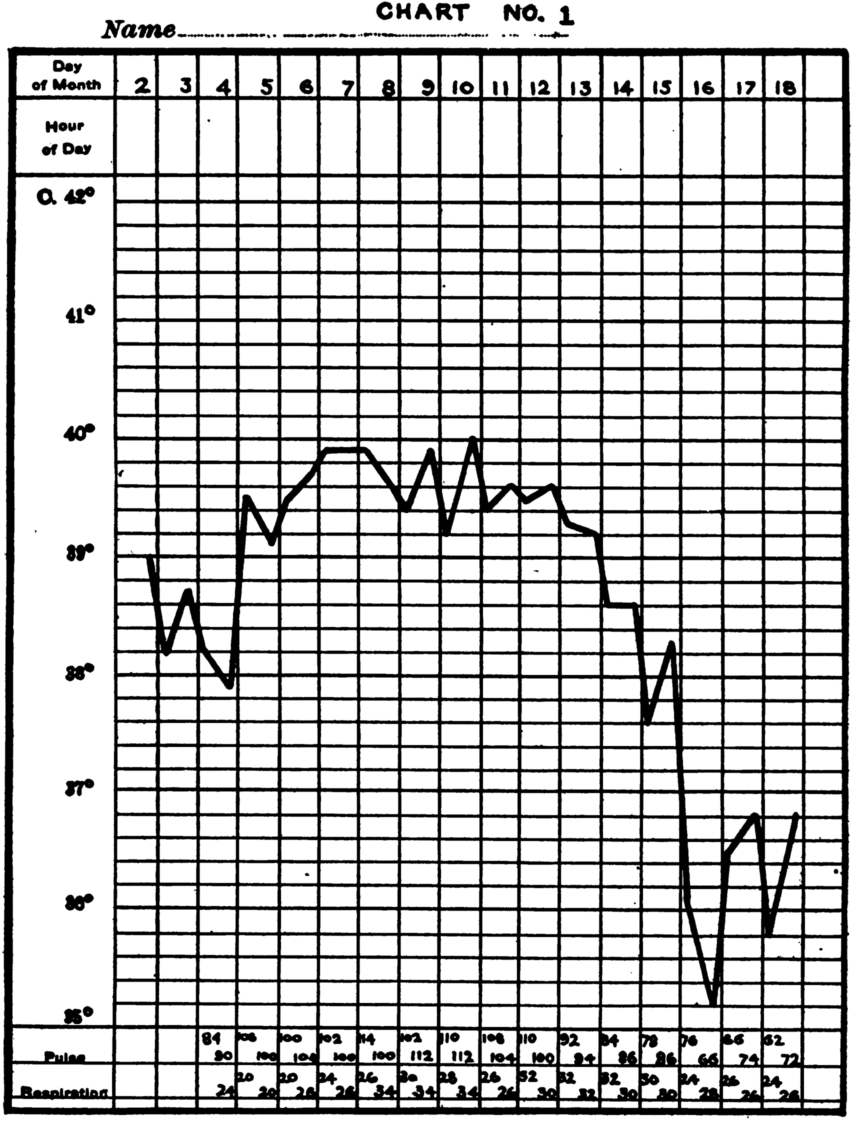
[1] Ricketts, H. T., and Wilder, Russell M.: The typhus fever of Mexico (tabardillo). Journ. Am. Med. Assn., vol. 54, Feb. 5, 1910, p. 463.

Having satisfied ourselves that neither contagion in the ordinary sense nor that fomites as usually understood played any rôle[1] in the transmission of the disease, and with the impression strongly in our minds that some intermediary host was the probable trans-

CHART NO. 1



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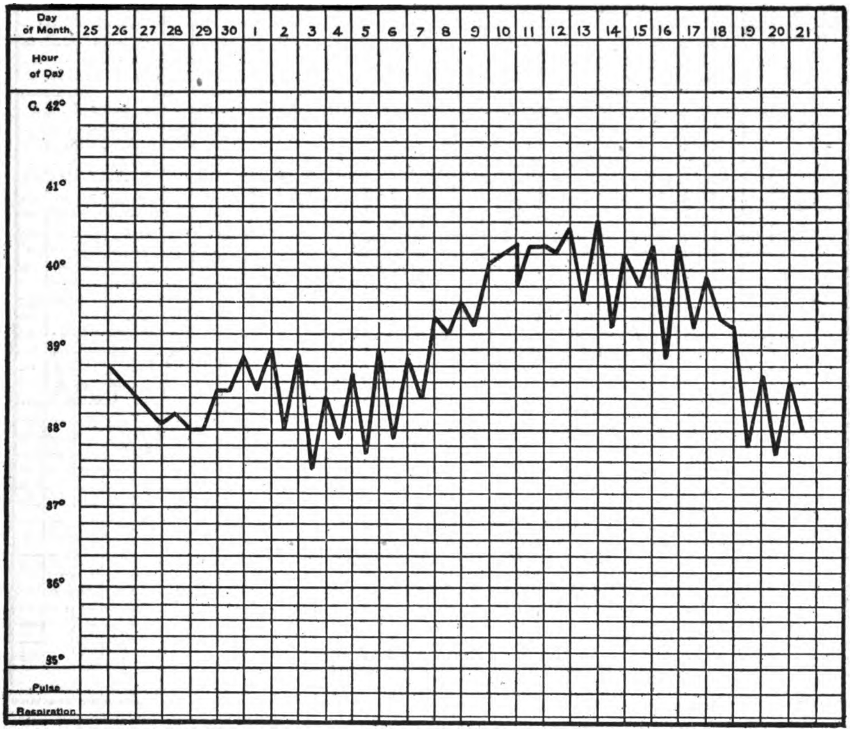
[1] As a matter of fact, we believe that both contact with individuals and fomites play a rôle in this disease, but only in the sense that by contact with individuals and fomites (lice-infested clothes or bedding) the transfer of the louse to another individual is favored.

mitting agent of the infection, we took up a consideration of the insects which might be expected to play this rôle. The insects which immediately suggested themselves as worthy of serious attention in this connection were the flea, the bedbug, and the body louse.

### THE FLEA.

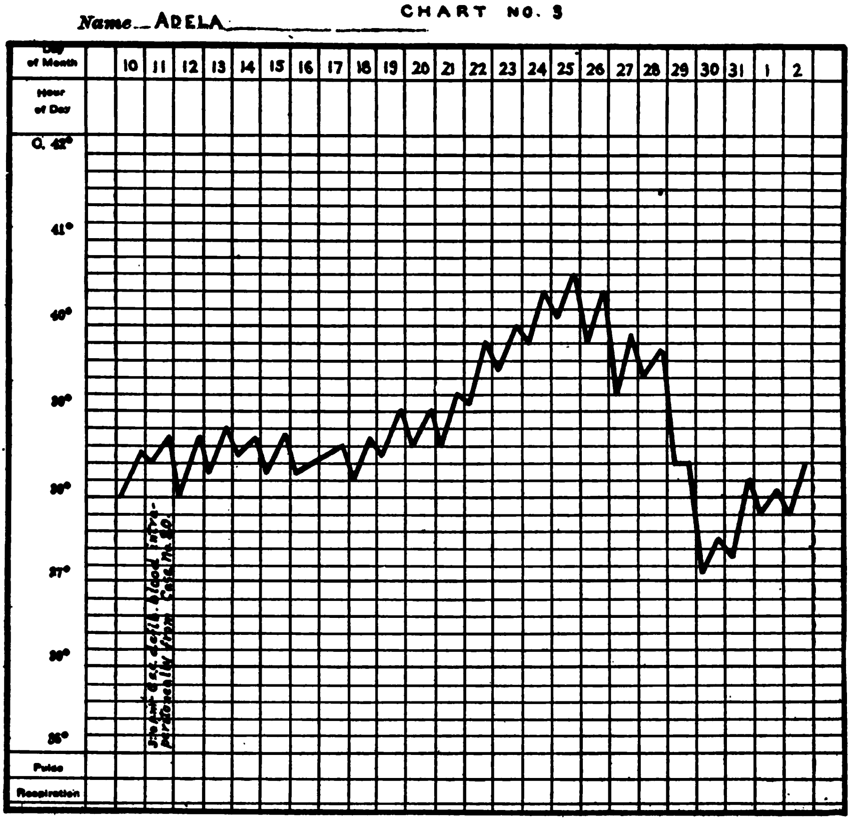
A review of certain facts enabled us very quickly to eliminate the flea from consideration. The ubiquity of the flea and the well-recognized limitation of the prevalence of the disease in the lowest social classes obviously do not harmonize with the idea that this insect is the transmitting agent. It is a well-recognized fact that in houses and families of the better classes secondary cases are rarely observed, though far from being free from infestation by fleas. Furthermore, it is a well-recognized fact that the striking distance of the infection is decidedly circumscribed, a fact with which the agility of the flea also does not harmonize.

Name Bedalia CHART NO. 2



### THE BEDBUG.

This insect is only a temporary parasite of man. As is well known, it lurks and hides in cracks and crevices and in the folds of bedding during the day, coming out at night to feed, retiring again toward morning. It is only rarely that it remains on the body of an individual. With this in mind, and assuming for the moment that the bedbug is the carrier, tabardillo should have the characteristics of a house disease, i. e., the infection should appear to be attached to houses and practically not at all to individuals, such as is the case in yellow fever. As a matter of fact, however, such is not the case. The distribution of the bedbug among the social classes is much more general than tabardillo, which, in a general way, is fairly limited to the poverty-stricken portion of the population. These facts appear to us to rule out this insect as the probable transmitter of tabardillo.



Finally, both the bedbug and the flea seem to us to be pretty definitely ruled out of consideration by the further fact that these insects are found in the lowlands of Mexico, where the disease is unknown except as isolated imported cases.

### THE BODY LOUSE.

Having eliminated the flea and the bedbug as the probable transmitters of the disease, there remains for consideration only the body louse (*Pediculus vestimenti*). This insect, as is well known, is parasitic on man, but may be said never to occur on persons or classes of people of cleanly habits. This fact may be seen very clearly in Mexico and is in entire harmony with the distribution of the disease among the social classes.

Students of the epidemiology of typhus fever have long observed that the disease, while apparently highly contagious, requires a fairly intimate contact for this contagiousness to manifest itself. Now, the body louse is a relatively sluggish insect. It practically makes only short excursions in the seams of the clothing of the individual and from these to the body of its host to feed. Its transfer from person to person takes place only when individuals are in close association with one another or with infested clothes or when the insect is accidentally dislodged from the body of its host and is subsequently picked up, as it were, by another person.

Impressed by these considerations we attempted to transmit the disease by means of the body louse, *Pediculus vestimenti*, from man to monkey. For this purpose we obtained a considerable number of these insects and distributed them in small wide-mouthed bottles, carefully stoppered. Two of these bottles were kept, during the day, at a temperature of 24° to 27° C., and at night at approximately 18° to 20° C. These lice were allowed to feed repeatedly upon the monkey "Jane," to which reference will shortly be made. After 21 days three survivors were killed and preserved. Two other bottles, containing some of the same original collection of lice, were kept day and night close to the body of one of us, with the idea that this was a more suitable temperature for them. At the end of 36 hours, although having received a feeding during this interval, all the insects were found dead.

At this stage of our work, Nicolle's[1] papers first came to our attention. In one of these he reported the successful transmission of typhus fever, in Tunis, from monkey to monkey, the *Macacus sinicus*, by means of the body louse, and we noted that his lice between feedings were kept at a temperature of 16° to 20° C. This suggested to us that probably the cause of the death of the lice in the two bottles which we had kept continuously at approximately body temperature was due to too high a temperature. We decided, therefore, to repeat the experiences of keeping the lice at approximately body temperature by placing another batch of these insects in two flasks and keeping them close to the body day and night. The result of this experiment was practically identical with our first experience. Only a few of the insects survived 48 hours, although, as in our first experience, they had been fed in the interval.

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[1] Nicolle, Ch.: Reproduction expérimentale du typhus exanthematique chez le singe; Compt. Rend. Acad. des Sciences, vol. 149, July 12, 1909, p. 157.

Nicolle, Ch., Comte, C., and Conseil, E.: Transmission expérimentale du typhus exanthematique par le pou du corps. Compt. Rend. Acad. des Sciences, vol. 149, Sept. 6, 1909, p. 486.

Several batches of the same collection of lice as were used for this experiment were kept at a temperature of 14° to 20° C. They were allowed to feed daily, and among these the mortality was comparatively slight. This influence of temperature on the longevity of the louse seems to us strikingly significant in relation to the apparently singular limitation of the disease to the Mexican plateau, i. e., to places having an altitude of not less than 1,500 to 1,800 meters, and its complete absence from lower altitudes, and in relation to its seasonal prevalence.

The peculiar limitation of the disease to places at a considerable altitude, as just stated, had been engaging our attention, and with the idea of the possibility that the body lice in places in the lower altitudes were perhaps of a different species from those on the plateau, we endeavored to obtain specimens in Tampico through the courtesy of the Rev. Neill E. Pressly, whose duties bring him into close and confidential relations with the natives. Replying to our communication, Mr. Pressly informed us that the body louse was practically unknown in that locality. Subsequently this was confirmed by Dr. Rolph, a physician located on a large ranch near Tampico. This gentleman, in conversation with us, stated that body lice did not exist in his locality, though head lice were common. He added, further, that this was so well known among the natives that when peons from the plateau in search of work arrived infested with body lice, they declined to take any special measures for the destruction of these parasites on the ground that the lice could not live there more than a few days, and such he actually found to be the case.

As already stated, we attempted to transmit the disease from man to monkey by means of the louse *Pediculus vestimenti*. The lice used for this purpose were obtained from the clothing of persons of a class in whom the infection is common. It was, therefore, quite possible, and indeed probable, that some of these may have already been infected. Before applying them to the monkey in the course of the experiment, we permitted them to feed on cases of tabardillo.

Experiment No. 1: "Jane," a female *Macacus cynomolgus*, was repeatedly exposed to the bites of several batches of lice obtained as above described. These lice, before being applied to the monkey and at various intervals in the course of the experiment between November 28 and December 31, had bitten cases of tabardillo in the first stages of the disease. The lice were applied to this animal in all thirty times. During the first 16 days of the experiment a single batch of lice (the first) was used throughout, and was kept at a temperature during the day of 24° to 27° C. and at night at 18° to 20° C. The several batches of lice later used were kept at a temperature of 14° to 22° C. The animal was kept under observation for 25 days after being last bitten by the lice. Then, after an interval of 5 days, during which time the observation was suspended, for a further period of 12 days. Throughout the period of observation this animal showed no abnormal elevation of temperature or other indications of illness. This experiment appears to have resulted negatively.

We regret that we have been unable to test the susceptibility of this monkey to blood inoculation; but inasmuch as it is specifically more closely related to the *Macacus rhesus* than the capuchin monkey, both of which species we have shown to be susceptible to blood inoculation, it seems, *a priori*, reasonable to assume the existence of such susceptibility in this species.

Experiment No. 2: "Raquel," a female *rhesus*, was exposed on six successive days to the bites of lice which had been twice allowed to feed upon case No. 19, and the third time upon case No. 20. Eight days after the last exposure the evening temperature of this animal rose to 39.6° C.; the following day it was 39.1° in the morning and 39.6° in the evening. These temperatures were a little above the normal range for this animal, but on the tenth day after the last exposure (January 25) the temperature of the animal had dropped back to within the limits of its normal range.

After this we were obliged to suspend observations for five days, when they were again resumed. The temperature of the animal was normal, and it presented no indications of illness. Whether the slight rise in temperature here recorded was the beginning of a brief reaction or not we are unable to say.

## THE RELATION OF TABARDILLO TO EUROPEAN TYPHUS.

Several distinguished Mexican clinicians have raised the question as to the identity of tabardillo and European typhus, basing their doubts as to the identity on certain apparent clinical differences in the two diseases. Certain differences in the results of our work and that of Nicolle would seem to give some experimental support to this view. Nicolle has reported that he was unable to inoculate monkeys of the species *Macacus sinicus* by blood directly from the human subject, but was obliged first to pass it through a chimpanzee, whereas we have been able to infect not only a closely related species, the *Macacus rhesus*, but also a *Cebus capuchinus*, directly with blood from a human case of tabardillo.

It is interesting to note, however, that the mode of transmission of European typhus and tabardillo is probably the same. Nicolle has reported a successful experiment with the body louse, and both Ricketts and Wilder and ourselves have independently come to regard this insect with grave suspicion as playing the same rôle in the Mexican disease.

## SUMMARY AND CONCLUSIONS.

1. At least two species of monkeys, *Macacus rhesus* and *Cebus capuchinus*, are susceptible to direct inoculation with the blood from human cases of tabardillo.
2. One attack of the disease in the monkey produced by blood inoculation directly from man induces a definite immunity to a subsequent inoculation with virulent blood. The reaction following blood inoculation in monkeys "Bedalia" and "Jerry," reported in our second note, was therefore the first recorded experimental production of tabardillo in monkeys.
3. The blood from human cases of tabardillo is infective on at least the eighth day of the disease. It seems probable, however, that it will be found infective throughout the active febrile stage of the disease.
4. The blood from the monkey, *Macacus rhesus*, is infective by passage to a second monkey of the same species on at least the fifth and sixth days of the disease.
5. Diluted blood serum from a human case of tabardillo, when passed through a Berkefeld filter, failed, when inoculated into a monkey, to produce the disease.
6. The blood of a monkey of the species *Macacus rhesus* is infective, though its infectivity is somewhat attenuated, to a second monkey of the same species.
7. The disease is not conveyed by fomites, as such, nor is it contagious in the ordinary sense of the word.
8. The epidemiological facts of the disease, in our opinion, point unmistakably to an insect intermediary; and we believe that our observations point strongly to the body louse (*Pediculus vestimenti*) as this insect.
9. We are of the opinion that the evidence against the body louse as transmitter of tabardillo is sufficient to demand that prophylactic sanitary measures directed against this disease should take into consideration that insect.

# 4.—THE RELATION OF SO-CALLED BRILL'S DISEASE TO TYPHUS FEVER.[1]

## AN EXPERIMENTAL DEMONSTRATION OF THEIR IDENTITY.

By **John F. Anderson**, *Director Hygienic Laboratory*, and **Joseph Goldberger**, *Passed Assistant Surgeon.*

Typhus fever has not appeared in epidemic form in the United States since the comparatively small outbreaks in New York in 1891–92 and in Philadelphia in 1883. Sporadic cases have been reported, as the four cases in Baltimore in 1901 and occasional cases among recently arrived immigrants. It has been a subject of speculation among health authorities why, in spite of the arrival of occasional cases in this country and of many persons from endemic foci of the disease, typhus fever apparently does not gain a foothold in the United States. It will be shown later that such a possibility is not without reason, and that the disease is actually present at all times in certain cities of the United States.

In 1898 Dr. Nathan E. Brill,[2] of New York, reported 17 cases of a disease clinically resembling typhoid fever but without the Widal reaction, and clearly set forth the differences between this group and the usual types of typhoid fever cases.

In April, 1910, he reported in a most interesting paper[3] a study of 221 cases of the same disease observed at Mount Sinai Hospital, New York City, from late in 1896 to December 1, 1909.

Since the important features of the disease as observed by him are well summed up in his definition, it is given:

> An acute infectious disease of unknown origin and unknown pathology, characterized by a short incubation period (four to five days), a period of continuous fever, accompanied by intense headache, apathy, and prostration, a profuse and extensive erythematous maculo-papular eruption, all of about two

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[1] Reprinted from the Public Health Reports, vol. 27, No. 5, Feb. 2, 1912.

[2] Brill, Nathan E.: A study of 17 cases of a disease clinically resembling typhoid fever, but without the Widal reaction; together with a short review of the present status of the serodiagnosis of typhoid fever. N. Y. Med. Journ., vol. 67, Jan. 8 and 15, 1898, pp. 48 and 77.

[3] Brill, Nathan E.: An acute infectious disease of unknown origin. A clinical study based on 221 cases. Am. Journ. Med. Sci., April, 1910.

**weeks' duration, whereupon the fever abruptly ceases either by crisis within a few hours or by rapid lysis within three days, when all symptoms disappear.**

In a third paper Brill[1] reports on the study of 34 additional cases observed since the 221 previously reported. It may be noted that these 34 cases all occurred in the year ended December 1, 1910, and in the same hospital, indicating an increasing prevalence of the infection or greater skill in diagnosis. This paper of Brill's is of especial interest and value because it includes, in addition to clinical data, the report of an autopsy on a fatal case and the results of the inoculation of monkeys with blood from two cases in the acute stage, and with the blood and emulsion of the liver and the spleen from the fatal case. Dr. Brill states that from the report of the autopsy "the conclusion may be drawn that the organs showed congestion and general parenchymatous degeneration such as would obtain in any intense infectious disease."

From material obtained at the autopsy, made about 18 hours after death, Brill inoculated three monkeys; one intravenously with 5 c. c. of heart's blood, one intraperitoneally with an emulsion of the liver, and one intraperitoneally with an emulsion of spleen. The animals were kept under observation for three months and showed no signs of illness.

Brill also reports inoculations with blood obtained during life. In one case 5 c. c. of blood was drawn from a patient in the seventh day of the disease and was inoculated subcutaneously and intraperitoneally into a *Macacus rhesus;* in a second instance 5 c. c. was drawn from a patient in the sixth day of the disease, diluted with 10 c. c. sodium citrate solution, and inoculated intravenously into a *semnopithecus.* The temperature of the first animal was taken for one month and of the second for three weeks. Both animals were kept under observation for two months, during which time they were entirely free from illness.

Brill states that this experimental work in inoculating monkeys has, at least so far, established a fundamental difference between the disease described by him and Mexican typhus fever sufficient, perhaps, to stamp them as separate diseases. He still reserves in his mind the belief that the disease may be due to an attenuated modification of the virus of typhus fever, and awaits the results of the further work of other investigators, although in a letter to the editor of the Journal of the American Medical Association[2] he apparently withdraws this reservation, as he states that he has in vain attempted to prove that it is typus fever and shall regard it as such only when certain evidence set forth in his letter is produced and that until then he believes he is justified in calling this disease "an infectious disease of unknown origin."

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[1] Brill, Nathan E: Pathological and experimental data derived from a further study of an acute infectious disease of unknown origin. Am. Journ. Med. Sci., August, 1911.

[2] Journ. A. M. A., vol. 57, Dec. 2, 1911, p. 1854.

Dr. Brill deserves the greatest credit for his careful study of the disease in question, and his ability as a close and careful observer is shown in first distinguishing cases of this disease, instances of which have almost certainly come under the observation of others.

Louria,[1] in a paper read before the Kings County Medical Society, reports the results of the observation of 18 cases in the Jewish Hospital, Brooklyn, N. Y., during the summer and fall of 1910.

Friedman,[2] on clinical grounds, expresses the opinion that Brill's symptom complex is identical with mild and moderately severe cases of typhus fever.

About the time that Brill's second paper appeared (April, 1910) we were engaged in the study of Mexican typhus fever[3] and, having the picture of that disease clearly in our minds, we were struck by the very marked clinical resemblance between it and the disease described by Brill. Influenced by this resemblance, we endeavored to obtain access to cases of Brill's disease in order to determine if possible the relationship between that infection and typhus fever. This we did not succeed in doing, however, until September, 1911.

Through the courtesy of Dr. Leo Kessel, New York, and of Dr. Olsan, house physician at Mount Sinai Hospital, New York, we had the privilege of seeing a case of Brill's disease in the wards of Mount Sinai Hospital on September 20, 1911. Chart No. 1 shows the temperature of this patient (case No. 1, N.[4]) from the day of his admission to the hospital to the day of his discharge. We shall not, at this time, go into the history of this patient beyond stating that he was in the seventh day of his illness when we first saw him, and that the case was considered by those familiar with Brill's disease to be a typical one of that disease.

Blood was drawn from the arm vein, defibrinated, and used for the inoculation of two rhesus monkeys (Nos. 149 and 150), each animal receiving 3 c. c. of the defibrinated blood intraperitoneally. The next day, in the eighth day of illness, blood was again drawn from an arm vein of this patient, defibrinated, and about three hours

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[1] Louria, Leon: Brill's disease. Med. Rec., Aug. 26, 1911.

[2] Friedman, G. A.: Brill's symptom complex; typhus fever; Manchurian typhus. Arch. Internal Med., vol. 8, Oct. 15, 1911, p. 427. Abst. Med. Rec., vol. 80, Sept. 16, 1911, p. 606.

[3] Anderson, John F., and Goldberger, Joseph: On the relation of Rocky Mountain spotted fever to the typhus fever of Mexico. A preliminary note. Public Health Reports, xxiv, Dec. 10, 1909, p. 1861.

Same: A note on the etiology of "tabardillo," the typhus fever of Mexico. Public Health Reports, xxiv, Dec. 24, 1909, p. 1941.

Same: On the infectivity of tabardillo or Mexican typhus for monkeys and studies on its mode of transmission. Public Health Reports, xxv, Feb. 18, 1910, p. 177.

Same: On the etiology of tabardillo or Mexican typhus. An experimental investigation, Journ. Med. Research, xxii, June, 1910, pp. 469–481.

[4] N.=New York series.

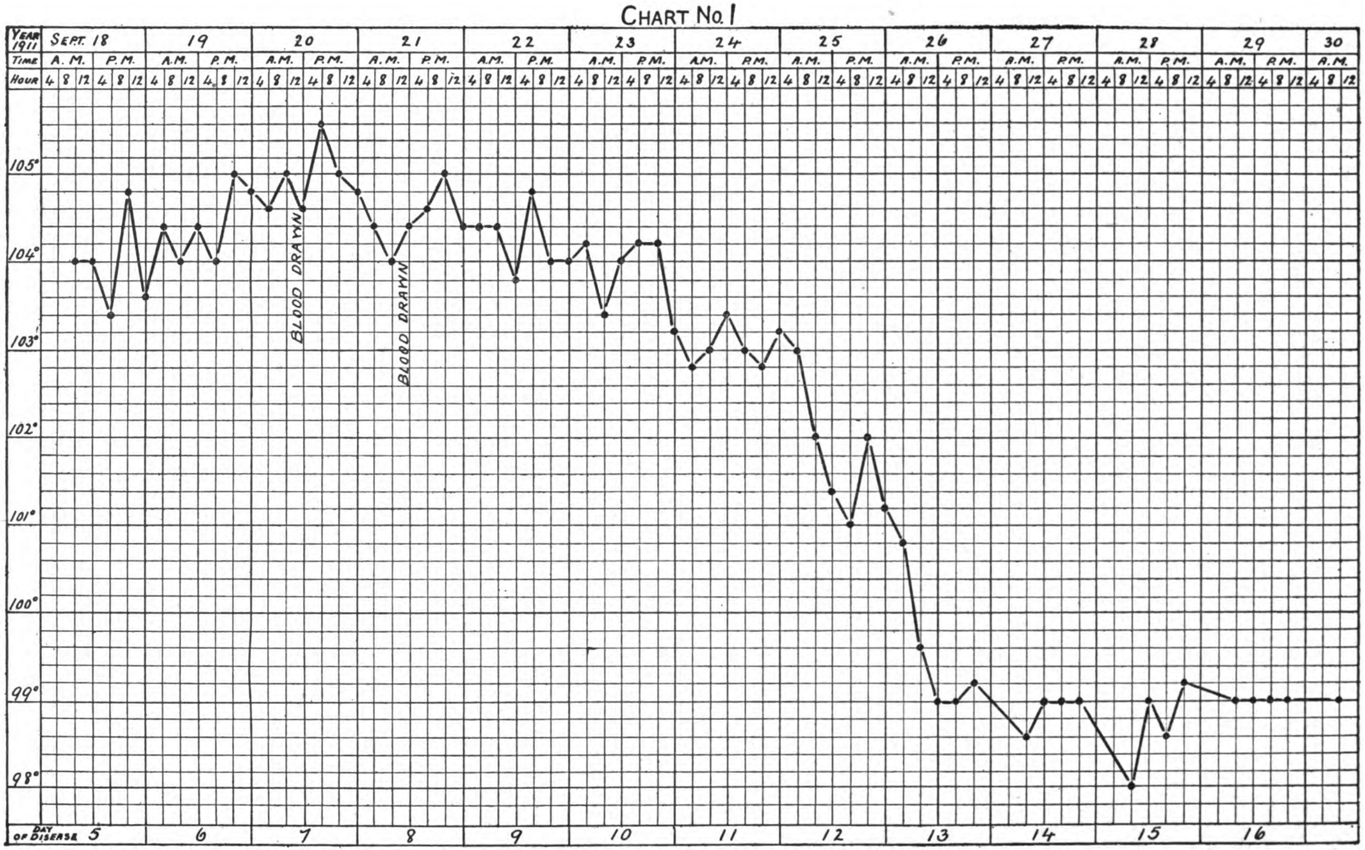

Chart No. 1, showing temperature of case No. 1 of "Brill's disease."

later was used for the inoculation of two rhesus monkeys (Nos. 151 and 152), each animal receiving approximately 3.5 c. c. of the blood, diluted with an equal volume of normal salt solution, intraperitoneally. Monkey No. 152 died 10 days later as a result of a purulent peritonitis, and monkey No. 151, which was in poor condition at the time of inoculation, and refusing food, continued progressively to fail. Six days later, being moribund, the animal was chloroformed.

Monkey No. 149, during a period of observation lasting 31 days, showed no evidence of illness, by rise of temperature or otherwise.

Ten days after inoculation the temperature of monkey No. 150 began to rise, reaching its maximum (41.2° C.) six days later. The temperature of the animal remained high until the eleventh day of fever, when it fell by rapid lysis, reaching normal 14 days after the rise began. On the sixth day of fever, when the temperature was at its maximum, 10 c. c. of blood were drawn from the animal's heart, defibrinated, and used for the inoculation of two rhesus monkeys. Rhesus No. 157 was given 3.5 c. c. of the defibrinated blood and rhesus No. 158 was given 3 c. c., both intraperitoneally.

Five-tenth cubic centimeter of the defibrinated blood were planted in a tube of standard bouillon, and during a period of observation of 12 days gave no evidence of growth.

The temperature of both animals (Nos. 157 and 158) began to rise on the ninth day after inoculation. As monkey No. 158 gave promise of a more severe reaction than monkey No. 157, the former was selected for passage.

On the afternoon of the ninth day after inoculation, and the first day of fever, when the temperature of the animal was 41° C., monkey No. 158 was bled from the carotid artery for 50 c. c. This blood was defibrinated and a portion used for passage. Since then this infection has been carried through 11 monkey generations by inoculation of blood.

Since our case No. 1 we have seen two more cases of Brill's disease. With blood obtained from a case in the Jewish Hospital, Brooklyn, we have succeeded in infecting a rhesus monkey and effecting passage with the blood of that animal.

After intraperitoneal or intravenous inoculation there is an incubation period of 5 to 14 days. At the end of this time there is usually a rapid rise of the animal's temperature, which frequently reaches a maximum in 36 or 48 hours of 41° to 41.5° C.; the temperature remains between 40° and 41° C. until toward the end of the febrile period, when it may show a gradual decline, but almost invariably declines by crisis or rapid lysis, frequently to subnormal. After about the second day of fever the animal may show other evidences of illness, such as loss of appetite, ruffled fur, crouching posture in cage, loss of weight, and weakness. Convalescence is usually rapid:

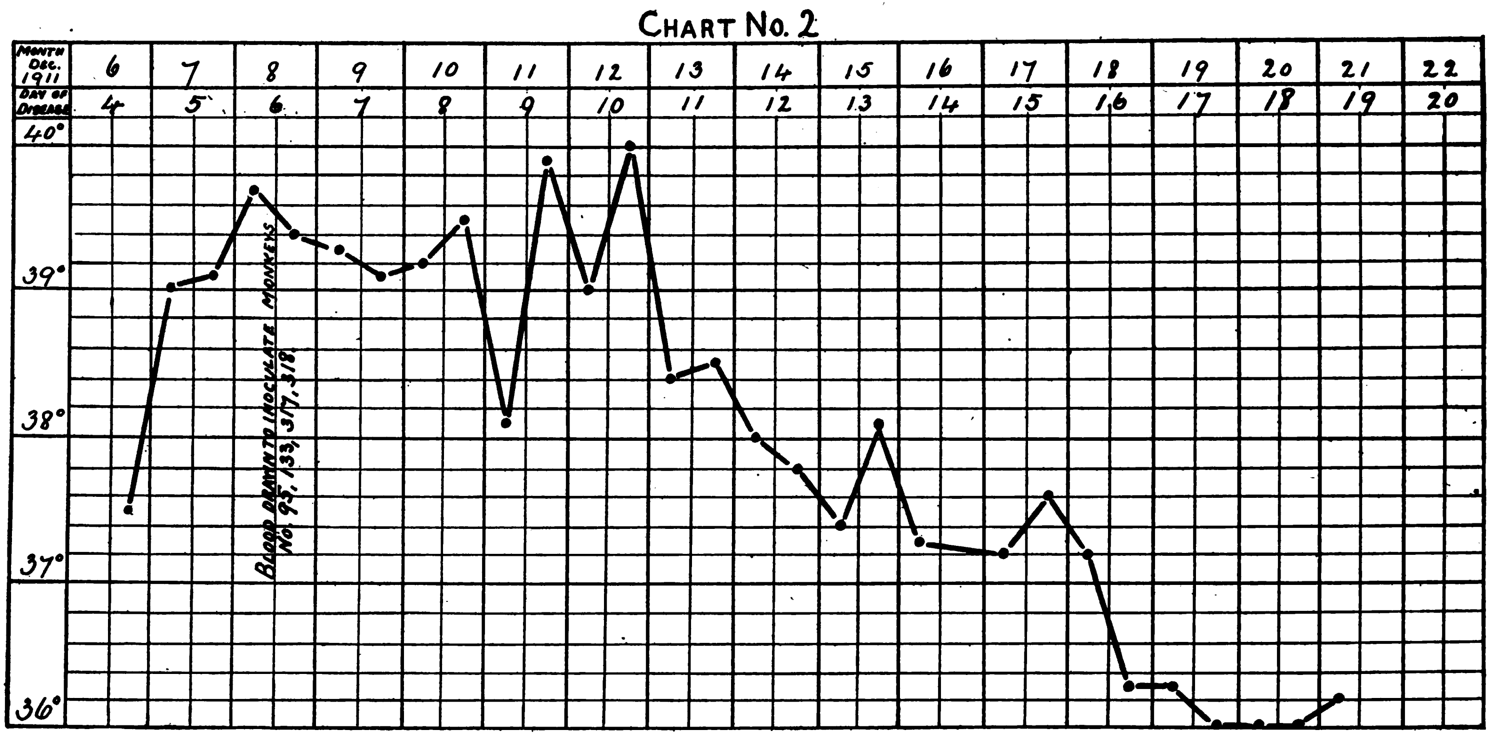

Chart No. 2, showing temperature of case No. 19 of typhus fever.

We have never noticed the presence of an eruption, although we have not searched very carefully for it. Occasionally one of a pair of animals may fail to develop the infection when inoculated with virulent blood, but on subsequent inoculation has invariably been found to be susceptible.

One attack of the infection has proved in every instance tested to have conferred immunity to infection by subsequent inoculation with virulent blood.

Having established the susceptibility of the rhesus monkey to infection by the inoculation with blood from a case of Brill's disease, it became important to determine the relationship of Brill's symptom complex to typhus fever. In order to clear up this question one of us (J. G.) proceeded to Mexico City, carrying a number of rhesus monkeys, some of which had recovered from infection with the virus obtained originally from our case No. 1 of Brill's disease, and others—fresh animals—to be used as controls.

On November 23, 1911, monkeys Nos. 95 and 133, which had recovered from an infection with the virus of Brill's disease, and control (normal) monkey No. 314 were each inoculated intravenously with 3 c. c. of defibrinated blood from rhesus No. 306. The latter animal was at this time in the third day of what appeared to have been a mild typhus reaction. During the succeeding 29 days rhesus No. 314 gave no evidence of a reaction.

On December 8, 15 days after the first inoculation, none of the three animals (Nos. 95, 133, and 314) having given any indication of a reaction, monkeys Nos. 95 and 133 were reinoculated with defibrinated blood from case No. 19–M,[1] a severe case of typhus in the sixth day of the disease (see chart No. 2). Two normal monkeys, Nos. 317 and 318, were similarly inoculated as controls. Each of these four animals was given 3.5 c. c. of the blood diluted with an equal volume of saline solution intraperitoneally.

*Result.*—Both control monkeys, Nos. 317 and 318, have given clear evidence of infection (see charts Nos. 3 and 4), whereas neither Nos. 95 nor 133 has given any indication of a reaction (see charts Nos. 5 and 6).

It might be suggested that the failure of monkeys Nos. 95 and 133 to react to the second typhus inoculation was due rather to a resistance conferred by the inoculation of November 23 with the blood of rhesus No. 306 than to an immunity following the attack of Brill's disease. That this is not the case, however, is evidenced by the fact that rhesus No. 314, originally inoculated as the control for Nos. 95 and 133 in their first test, made on November 23, has since reacted promptly and sharply following a second inoculation with blood from a human case of typhus.

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[1] M=Mexican series.

**CHART No. 3**

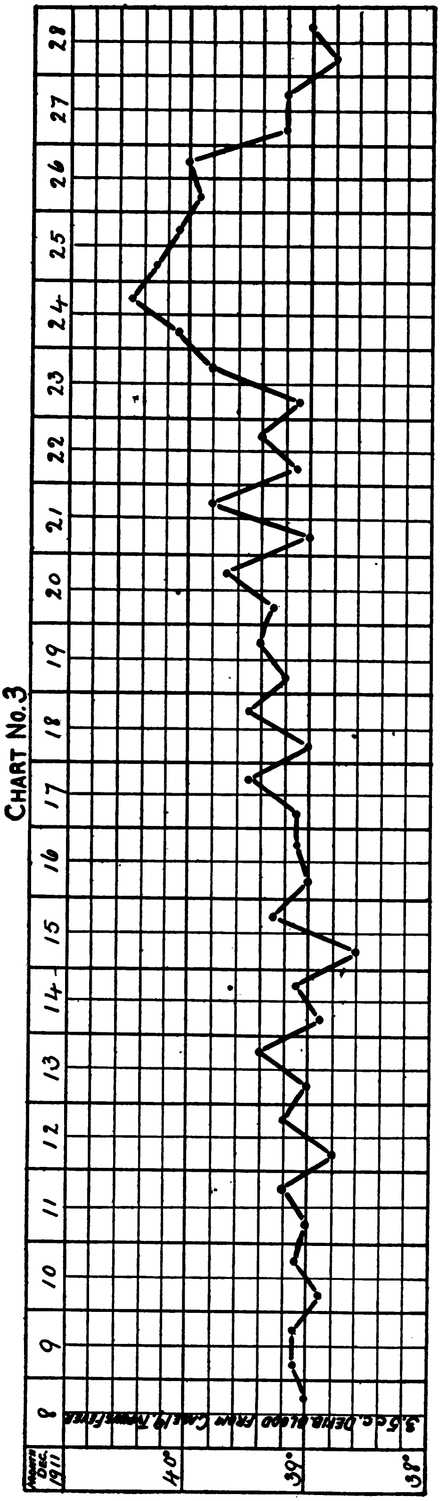

Chart No. 3, showing course of temperature of monkey No. 317; see also charts Nos. 5 and 6.

In order to leave no room for doubt on this point we inoculated two other monkeys (Nos. 161 and 162) that had previously reacted to an inoculation with the virus of Brill's disease. On December 29, 1911, these two animals were inoculated intraperitoneally with 6 c. c. each of defibrinated blood from a well-marked case (case 35–M) in the ninth or tenth day diluted with an equal volume of saline solution. January 1, 1912, both these animals were reinoculated with defibrinated blood from another patient (case 39–M) in the ninth day of disease, each animal receiving 4 c. c. diluted with an equal volume of saline solution intraperitoneally. Neither animal has given evidence of a reaction, although two other monkeys (Nos. 304 and 324), inoculated in precisely the same way and on the same dates, have reacted promptly and sharply.

We believe, therefore, that the conclusion is justified that an attack of Brill's disease in the monkey confers an immunity to subsequent infection with Mexican typhus (tabardillo).

Monkeys Nos. 317 and 318, which had passed through an attack of Mexican typhus fever and which has served as controls in the immunity test above described, were tested for their immunity to infection derived originally from a case of Brill's disease. These two monkeys, as well as control, were given 3 c. c. of defibrinated blood from rhesus No. 187, then in the second day of an attack of Brill's disease.

*Result.*—The control monkey has given a definite reaction, while neither No. 317 nor No. 318 has given any indication of a reaction. It is, therefore, permissible to conclude that Nos. 317 and 318, as a result of their attack of Mexican typhus fever, are immune to infection with Brill's disease.

## SUMMARY AND CONCLUSIONS.

1. The rhesus monkey is susceptible to infection by inoculation with the blood from a case of "Brill's disease."

2. One attack of the disease in the monkey induces a definite immunity to a subsequent infection with virulent blood of the same strain.

3. Monkeys recovered from an infection with "Brill's disease" have been found to be immune to a subsequent infection with virulent blood from a case of Mexican typhus fever.

4. Monkeys recovered from an infection with Mexican typhus fever have been found to be immune to a subsequent infection with "Brill's disease."

5. From the above results we conclude that the disease

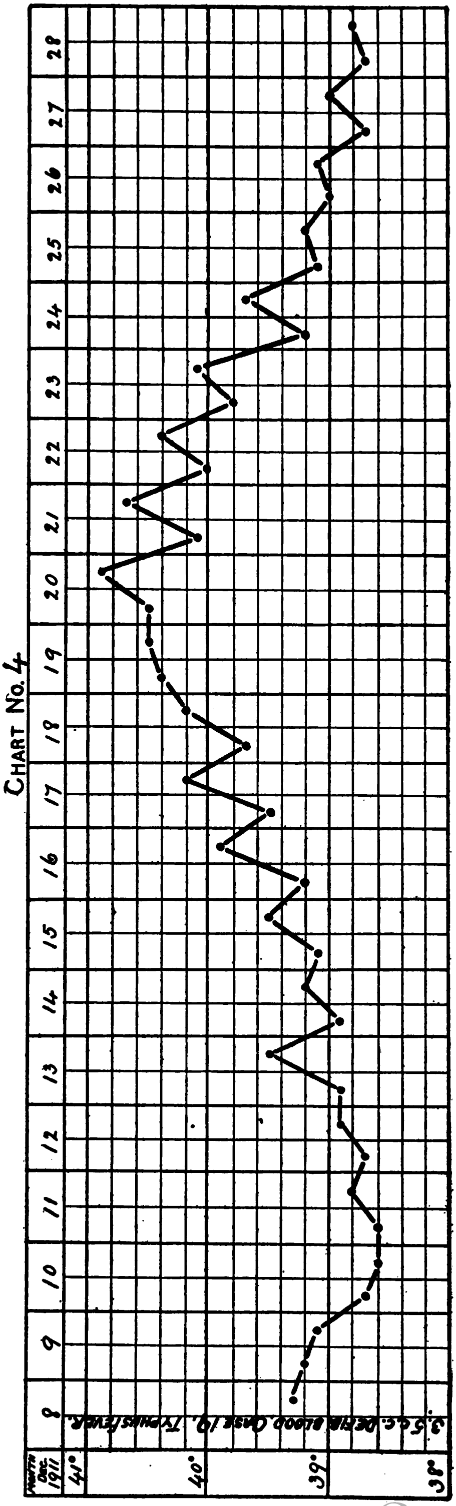

Chart No. 4, showing course of temperature of monkey No. 318; see also charts Nos. 5 and 6.

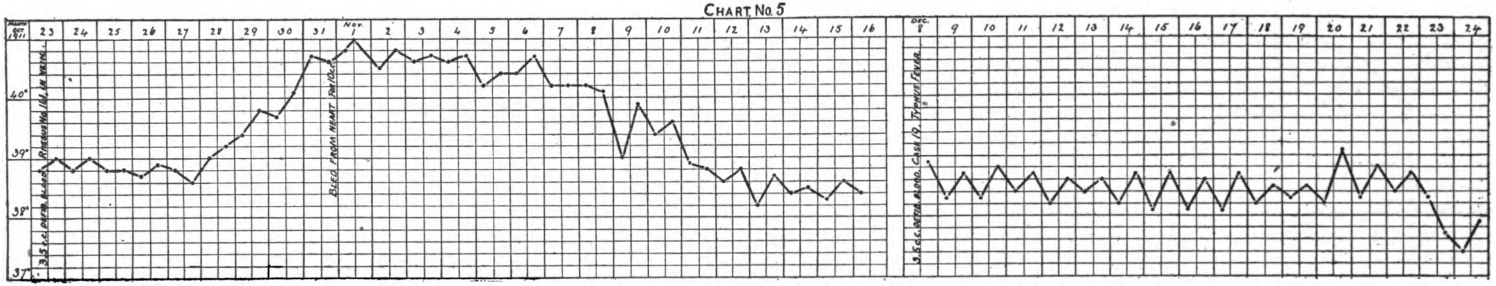

Chart No. 5, showing course of temperature of monkey No. 95. The chart shows the temperature curve during the attack of "Brill's" disease and the negative reaction following test of immunity by injection of blood from typhus fever case No. 19; see also charts Nos. 3 and 4.

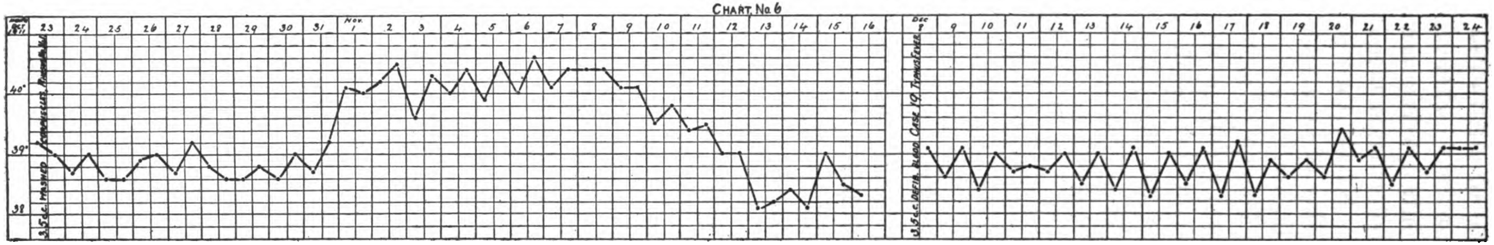

Chart No. 6, showing course of temperature of monkey No. 133. The chart shows the temperature curve during the attack of "Brill's" disease and the negative reaction following test of immunity by injection of blood from typhus fever case No. 19; see also charts Nos. 3 and 4.

described by Brill is identical with the typhus fever of Mexico, and inasmuch as the New York strain is undoubtedly of European origin, we may also conclude that the typhus of Europe and the tabardillo of Mexico are identical.

6. If this conclusion is correct, typhus fever has been present in New York City for a number of years and, according to verbal reports made to us, has occurred in other large cities of the United States.

7. These results make the clinical recognition and study of typhus fever of increased importance and necessitate the exercise of appropriate prophylactic measures.

8. It is not intended to exaggerate the menace of this disease to the public health. Nevertheless, although the disease in New York City has apparently been mild and has shown little tendency to spread, it is apparently on the increase there, and the possibility should be borne in mind that it may acquire virulence and epidemic prevalence.

We desire to express our indebtedness and to extend our thanks to Dr. Nathan E. Brill and Dr. Leo Kessel, of New York; Dr. Hiram Olsan, house physician at Mount Sinai Hospital, New York; Dr. Leon Louria, Brooklyn; and to Dr. Ellis Campus, house physician at the Jewish Hospital, Brooklyn, for the privilege of seeing cases of "Brill's disease" and obtaining material therefrom, and for many other courtesies.

For the laboratory and clinical facilities freely extended to us in Mexico City we are deeply indebted to Dr. Eduardo Licéaga, president of the superior board of health; to Dr. Octaviano Gonzalez Fabella, director of the bacteriological laboratory; to Dr. Regino Gonzalez, director of the Hospital General; and to Dr. German Diaz Lombardo, director of the Hospital Juarez. We are also under many obligations to Dr. A. R. Goodman, chief surgeon of the Mexican Railways, for numerous helpful courtesies. For their kind generosity in permitting us to study their cases our thanks are due to Drs. Miguel Otero and Zenón Luna.

# 5.—THE TRANSMISSION OF TYPHUS FEVER, WITH ESPECIAL REFERENCE TO TRANSMISSION BY THE HEAD LOUSE "PEDICULUS CAPITIS."[1]

By JOSEPH GOLDBERGER, *Passed Assistant Surgeon*, and JOHN F. ANDERSON, *Director Hygienic Laboratory, Public Health Service.*

In a recent paper[2] we showed that Brill's disease, which is apparently endemic in New York City, is identical with the typhus fever of Mexico, and as it is reasonably certain that the New York disease is of European origin we also concluded that the typhus of Europe and that of Mexico are identical. During the progress of the experiments necessary for the foregoing demonstration of the identity of the so-called Brill's disease and Mexican typhus we took up the study of various related problems. These studies were carried out concurrently in Mexico and at the Hygienic Laboratory in Washington.

At this time we desire to present the results of some of our work on the mode of transmission of this disease. Before presenting the details of this work it will be desirable to briefly review the present status of our knowledge of the transmission of typhus fever.

Nicolle, Comte, and Conseil[3] were the first to demonstrate insect transmission of typhus fever. In September, 1909, they reported the successful transmission of typhus fever from one bonnet monkey (*M. sinicus*) to two others by means of the body louse (*Pediculus vestimenti*). In this paper they showed that body lice that had fed upon an infected monkey were able to convey typhus fever some time between the first and the seventh day thereafter.

Independently of Nicolle, Comte, and Conseil, Anderson and Goldberger,[4] beginning their work on this problem in November, 1909, reported two attempts to transmit Mexican typhus from man to monkey by means of the bite of the body louse in February, 1910. In one of their experiments one of the monkeys showed a slight elevation of temperature eight days after the last exposure to the bites of the infected lice. Unfortunately, circumstances prevented their making an immunity test; but in the light of later studies it seems probable that the elevation of temperature was due to infection with typhus.

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[1] Reprinted from the Public Health Reports, vol. 27, No. 9, Mar. 1, 1912.

[2] Anderson, John F., and Goldberger, Joseph: The relation of so-called Brill's disease to typhus fever. An experimental demonstration of their identity. Public Health Reports, vol. 27, Feb. 2, 1912, p. 149.

[3] Nicolle, Charles; Compte, C.; and Conseil, E.: Transmission expérimentale du typhus exanthematique par le pou du corps. C. R. des Acad. Sci., vol. 149, Sept. 6, 1909, p. 486.

[4] Anderson, John F., and Goldberger, Joseph: On the infectivity of tabardillo or Mexican typhus for monkeys and studies on its mode of transmission. Public Health Reports, vol. 25, Feb. 18, 1910, p. 177.

Ricketts and Wilder,[1] also working in Mexico, reported in April, 1910, that they were able to transmit the virus of typhus fever by means of the bite of the body louse from man to monkey and from monkey to monkey. They also reported the successful transmission of typhus virus to the monkey by introducing into scarifications of the skin the abdominal contents of some infected lice. They record the interesting observation that, as a rule, in their louse experiments, the monkeys had no very significent temperature reaction and that proof of infection was dependent upon immunity tests with virulent blood.

In July, 1911, Wilder[2] reported additional experiments on infection of monkeys with body lice by biting and intradermal inoculation.

In January, 1911, Nicolle and Conseil,[3] continuing their work of September, 1909, report further successful experiments on the transmission of typhus fever to the bonnet monkey by means of the bite of infected body lice.

## EXPERIMENTAL.

### BODY LICE (PEDICULUS VESTIMENTI).

The first experiments we wish to report were made with body lice.

*Experiment No. 1.*

A number of body lice were collected from the clothes of healthy persons and on the afternoon of October 18, 1911, were applied to the belly of rhesus No. 158. They were again allowed to feed on No. 158 in the morning and afternoon of October 19, and again in the morning of October 20. In the afternoon of October 20 and thereafter twice daily up to and including October 22 they were fed on rhesus No. 157. Rhesus No. 157 and No. 158 were both sick at this time with typhus fever (New York virus—Brill's disease). After having fed in diminishing numbers on five successive days on sick monkeys the lice were applied to a fresh monkey, rhesus No. 127, in the morning and afternoon of October 23 and twice daily thereafter up to and including October 29, when feeding was discontinued. At this time 22 of the lice were still alive and were used in experiment No. 2. During the experiment the lice were kept at a temperature of 15° to 18° C.

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[1] Ricketts, Howard T., and Wilder, Russell M.: The transmission of the typhus fever of Mexico (tabardillo) by means of the louse (*Pediculus vestimenti*). Journ. Am. Med. Assn., vol. 54, Apr. 16, 1910, pp. 1304–1307.

[2] Wilder, Russell M.: The problem of the transmission of typhus fever, Journ. Infec. Dis., vol. 9, July, 1911, pp. 9–101.

[3] Nicolle, Charles, and Conseil, C.: Etiologie du typhus exanthematique. Ann. de l'Inst. Pasteur, vol. 25, pp. 68–78.

Rhesus No. 127 was kept under observation for 37 days after the last feeding, when the animal was given an immunity test by inoculation with virulent blood. After 9 days incubation rhesus No. 127 developed typhus fever, indicating that the animal had not been infected by the previous biting of the lice.

### *Experiment No. 2.*

The lice used in experiment No. 1 were killed with chloroform vapor on October 30, the day after their last feed on rhesus No. 127. They were then ground in a mortar with salt solution and injected subcutaneously into rhesus No. 137. Forty-three days later, having in the meantime given no evidence of infection with typhus fever, this monkey was given an immunity test consisting of an injection of virulent blood (New York virus). To this it responded after nine days' incubation with a typical attack of typhus fever, showing that the injection of crushed lice killed with chloroform vapor had not infected rhesus No. 137 with typhus.

### *Experiment No. 3.*

On October 27 about 150 body lice were obtained from the clothes of healthy persons and applied to the belly of rhesus No. 139 in the afternoon of the same day. Twice daily thereafter up to and including the morning of October 31 they were allowed to feed on rhesus No. 139. In the afternoon of October 31 and in the morning and afternoon of November 1 they fed on rhesus No. 95. Rhesus Nos. 139 and 95 were at this time both sick with typhus, induced by blood inoculation with the New York virus (Brill's disease). From November 2 up to and including November 9 they were fed twice daily on rhesus No. 165, a fresh monkey. When the feedings were discontinued on November 9 only nine lice remained alive. Throughout this experiment the lice were kept at 15° to 18° C. Chart No. 1 shows the temperature curve of rhesus No. 165 from the first feeding by infected lice on November 2 up to the time of the animal's death. Eleven days after the first and four days after the last feeding by infected lice the temperature of rhesus No. 165 began to rise and remained elevated four days, when it fell below its normal range. Ten days later the animal apparently had a relapse lasting three days. From this time on the animal progressively failed, and death occurred on December 8. At the autopsy

CHART No. 1

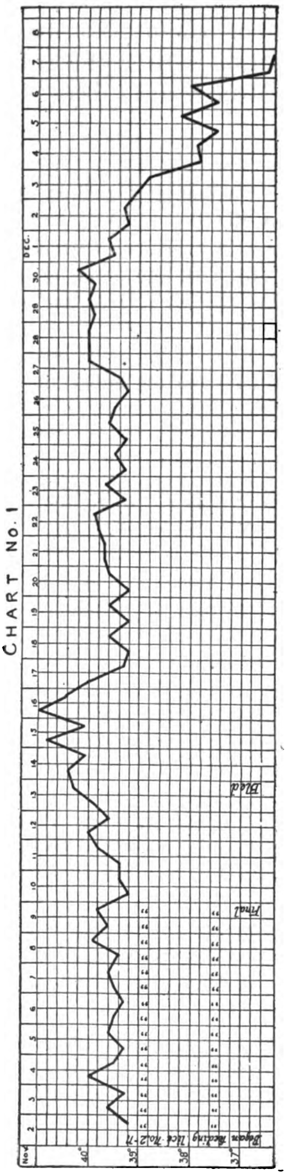

CHART No. 1.—Temperature curve of rhesus No. 165, infected by bites of body lice No. 2–N.

no macroscopical changes were noted in any of the organs. An attempt at passage on the first day of what we interpret as typhus fever, with the blood of this animal, using washed corpuscles, failed.

In spite of the failure at passage (compare experiment No. 7 below), we believe it is permissible to conclude that the rise in temperature of rhesus No. 165, beginning on November 13, was due to infection with typhus (Brill's disease), following the bite of infected lice from 4 to 11 days previously.

### *Experiment No. 4.*

On November 2, 6 days after their first and 1 after their last infecting feed, 10 of the lice used in experiment No. 3 were killed with chloroform vapor, ground in a mortar with salt solution, and injected subcutaneously into rhesus No. 145. Having given no evidence of a constitutional reaction, the immunity of this animal was tested 40 days after the injection of the crushed lice by an injection of virulent typhus blood (New York virus). The monkey developed fever after 8 days' incubation, indicating that infection with typhus had not resulted from the subcutaneous injection of crushed lice killed with chloroform vapor.

### *Experiment No. 5.*

About 9.30 a. m. on December 3, 83 body lice (*Pediculus vestimenti*) of group No. 7-M[1] and 83 of group No. 8-M were mixed, crushed in a mortar, and ground up in saline solution. Of this suspension 3.5 c. c., representing about 35 body lice, was subcutaneously injected into rhesus No. 308 and 1.5 c. c., representing about 15 lice, into rhesus No. 309.

Body lice of group No. 7-M had been allowed to feed daily during the 6 days immediately prior to the date of the experiment on various cases of typhus fever in the Hospital General, Mexico City. They were last applied to case No. 16-M, in the eleventh day of illness, at 10.50 to 11.20 a. m. December 2.

Body lice of group No. 8-M were insects that had been allowed to feed daily during the 5 days immediately preceding the date of the experiment on various cases of typhus fever. They were last applied to case No. 16-M, in the eleventh day of illness, at 10.20 to 10.50 a. m. December 2. Throughout the experiment these lice were kept at room temperature (about 14° to 24° C.).

Following the foregoing inoculations a slight redness of the skin developed at the site of injection in rhesus No. 308. This was incised about 24 hours after inoculation, but no pus was found. In the case of rhesus No. 309 some redness and swelling developed at the site of injection, which was incised 24 hours after the injection and a small amount of pus evacuated. During the period of observation of both animals no evidence of a febrile reaction was noted in either.

On January 10, 1912, or 38 days after their inoculation with the crushed-lice suspension, having in the meantime been returned to the Hygienic Laboratory, the two monkeys were given an immunity test, each animal receiving 3 c. c. of defibrinated blood of rhesus No. 187 (New York virus) intravenously. Following this inoculation both animals reacted sharply, No. 308 after an incubation period of 8 and No. 309 of 9 days.

The inoculation of these two animals with the crushed-lice suspension had, therefore, been without appreciable result.

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[1] M=Mexican series; N=New York series.

### *Experiment No. 6.*

The preceding experiment was repeated on December 10, with 73 body lice (*P. vestimenti*) of group No. 9–M. The body lice composing this group were variously applied and fed on cases of typhus fever at the Hospital General, Mexico City, during the 6 days immediately prior to the date of the experiment. Throughout this period they were kept at room temperature (14° to 24° C.). They were last applied on December 9, between 10.15 a. m. and 12 m., to case No. 19–M, in the seventh day of illness. About 29 hours later, namely, December 10, at 5.30 p. m., they were crushed and rubbed up in saline solution and at 8.05 p. m. subcutaneously injected in rhesus No. 320. During the subsequent period of observation monkey No. 320 gave no evidence of a reaction.

On January 10, 1912, 31 days after the injection of the crushed-lice suspension, having in the meantime been returned to the Hygienic Laboratory, this animal was given an immunity test consisting of an injection of 3 c. c. of defibrinated blood of rhesus No. 187 (New York virus), part intravenously and part subcutaneously. Twenty-one days later, namely, January 31, having in the meantime given no indication of a reaction, he was given a second immunity test, consisting of 2.5 c. c. of defibrinated blood of rhesus No. 115–A (New York virus) intravenously. During a period of observation of 23 days following this inoculation no evidnce of a reaction developed. At each test a pair of fresh monkeys were inoculated as controls, and in each instance one of the respective pairs developed fever. Although only one of each pair of controls reacted, we believe it more than probable that the failure of monkey No. 320 to react was due to an immunity conferred by the previous injection with crushed-lice suspension.

## HEAD LICE (PEDICULUS CAPITIS).

Although all the important features of the epidemiology of typhus are satisfactorily explained on the basis of its transmission by means of the body louse (*P. vestimenti*), nevertheless, on account of the close relationship between this insect and the head louse (*P. capitis*), it seemed desirable to test the possibility of the transmission of the disease by this latter species.

Our first experiment with head lice was made to determine whether the virus of typhus was capable of retaining its virulence as long as 20 to 24 hours in the body of this insect.

### *Experiment No. 7.*

On November 14, 1911, 17 lice of group No. 1–M were crushed and rubbed up in saline solution and then, after a moment's standing to allow the coarse particles to settle, the suspension was injected subcutaneously into rhesus No. 306. The lice of group No. 1–M were obtained from the hair clipped from the scalp of case No. 4–M on November 12, on admission to the typhus ward of the Hospital General, Mexico City. At the time of admission this patient was in the eighth day of his disease. At 10 a. m., November 13, these insects were applied to a patient (case No. 1–M) in the thirteenth day of a well-marked attack and 15 of the insects fed. At 11.30 a. m., November 14, 12 of the 17 lice were still living. Both the living and the dead, 17 in all, were, as above recited, crushed and injected into rhesus No. 306. During the progress of the experiment the insects were kept at room temperature (14° to 24° C.).

On the following day, November 15, 23 lice of group No. 2–M, after being crushed and rubbed up as were those of group No. 1–M, were subcutaneously injected into the same monkey—rhesus No. 306. The lice of group No. 2–M were collected from the hair clipped from the scalp of a patient (case No. 5–M) in the eighth day of a well-marked attack of typhus at the time of admission to the typhus pavilion, namely, about 3.30 p. m., November 14, 1911. These head lice were kept at air temperature (14° to 24° C.) until 11.30 a. m. of the next day, when 13 were found alive and 10 dead. At this time, at least 20 hours from the last possible feed, these lice, both living and dead, were crushed and subcutaneously injected into rhesus No. 306. Following this injection there was practically no local reaction, but the temperature of the animal rose rapidly (see chart No. 2), reaching 41.1° C. on November 16. It dropped with equal rapidity to normal, where it remained until November 21. In the afternoon of this day, or 7 days after the first and 6 days after the second injection, the temperature of the animal again rose and remained elevated for 7 days. At the end of this period it declined rapidly to normal, where it has remained.

An attempt at passage from the monkey was made on November 23, about 48 hours after the second rise in temperature. Blood was aspirated from the heart, at once defibrinated, and 3 c. c. injected intravenously into rhesus No. 314, a fresh monkey; but during a subsequent period of 29 days this animal gave no evidence of a reaction. This failure to effect passage made it necessary to subject rhesus No. 306 to an immunity test in order to determine definitely the nature of the febrile reaction following the above inoculation with head lice.

On December 29, therefore, rhesus No. 306 was given an intraperitoneal injection of 6 c. c. of defibrinated blood from case No. 35–M, diluted with an equal volume of saline solution. Three days later the animal was given an additional intraperitoneal injection of 4 c. c. of defibrinated blood of case No. 39–M, likewise diluted with an equal volume of saline solution. Following these inoculations the temperature of the monkey has remained normal, though two others (rhesus Nos. 324 and 304) inoculated with identical quantities of the same specimens of blood on the same dates have given prompt and sharp febrile reactions, testifying to the virulence of at least one of the specimens used for their inoculation.

We conclude, therefore, that the febrile reaction beginning November 21 suffered by rhesus No. 306 was due to infection with the typhus virus in the bodies of the head lice with which it was inoculated.

Although the typhus virus may retain its virulence in the body of the head louse for at least 20 to 24 hours, as shown in the foregoing experiment, it does not necessarily follow, though strongly suggested, that this louse is capable of transmitting the disease in the normal way, namely, by biting. The following experiments with head lice were made, therefore, to test this point:

### *Experiment No. 8.*

On November 16, 1911, at 12 m., 25 head lice of group No. 3 were applied to the shaved belly of rhesus No. 302 and 15 of them fed. At 4.30 p. m. this group of lice was reapplied and again 15 fed. After this the lice were applied to rhesus No. 302 twice daily in rapidly diminishing numbers till November 19, in the afternoon of which date a solitary survivor was given its last feed.

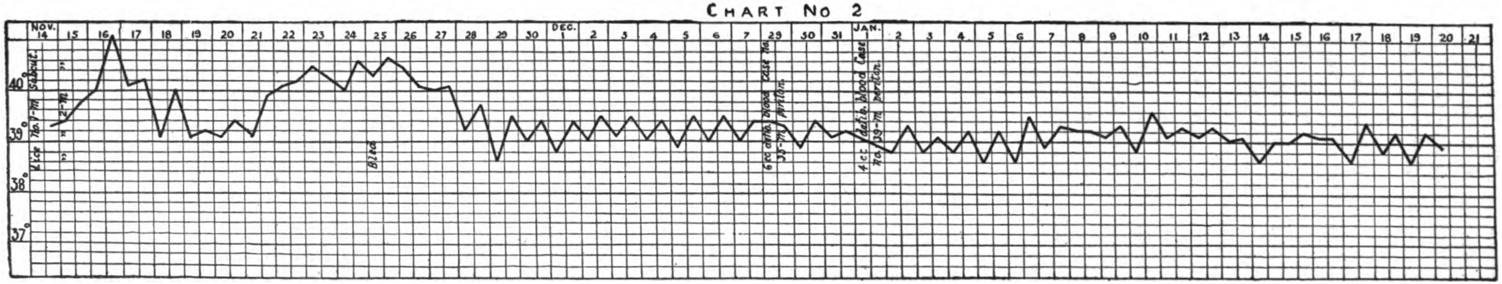

CHART No. 2.—Temperature curve of rhesus No. 306 after inoculation by subcutaneous injection of head lice No. 1-M and No. 2-M, and after immunity test.

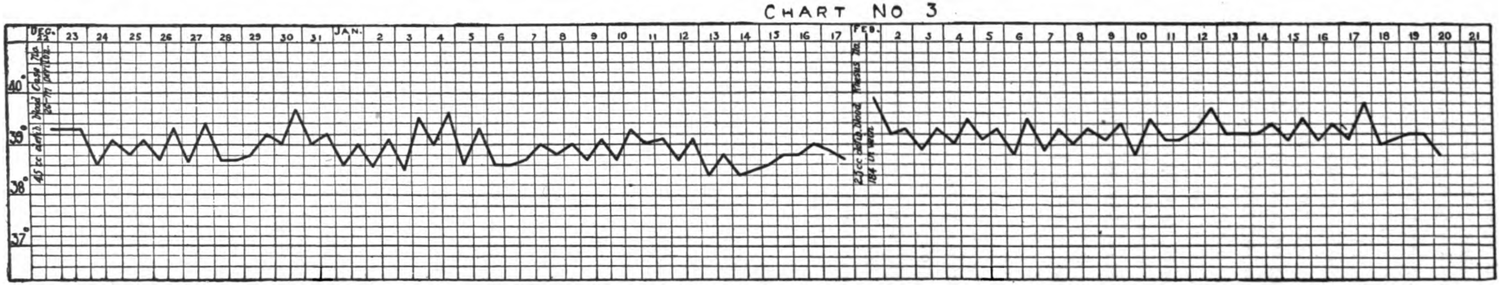

CHART No. 3.—Temperature curve of rhesus No. 302 after the first and the second immunity tests.

The lice of group No. 3–M were head lice collected from the heads of 3 typhus patients (cases 6–M, 7–M, and 8–M) in the afternoon of November 15, at the time of their admission to the typhus ward of the Hospital General, Mexico City. From the time they were collected these insects were kept at room temperature (about 14° to 22° C.).

In addition to the foregoing rhesus No. 302 was subjected to the bites of two lice constituting group No. 4–M in the forenoon and afternoon of November 18. Thereafter, twice daily, a single survivor of this group was applied until the morning of November 20, when this insect obtained its final feed. The two lice constituting group No. 4–M were obtained from the hair of the head of case No. 9–M at about 3 p. m. of November 17 on admission to the typhus ward. Two hours later they were reapplied to this patient and both fed. Following this they were kept at room temperature (about 14° to 22° C.).

During a period of observation of 30 days subsequent to the last inoculation by the bite of the survivor of lice group No. 4–M rhesus No. 302 gave no appreciable reaction. On December 22, or 32 days after being last bitten, this monkey was subjected to an immunity test by receiving an intraperitoneal injection of 4.5 c. c. of defibrinated blood of case No. 26–M, diluted with an equal volume of normal saline solution. At the same time and with some of the same blood, monkeys Nos. 314, 315, and 316 were similarly inoculated, Nos. 314 and 316 each receiving 4.5 c. c. and No. 315, 5 c. c., likewise diluted with equal volumes of saline solution. Of these 4 animals No. 302 was much the smallest, No. 315 very much the largest, while Nos. 314 and 316 were intermediate in size between Nos. 302 and 315. It follows therefore, that although Nos. 314 and 316 received the same amount of blood as No. 302 and No. 315 about 0.5 c. c. more, rhesus No. 302 actually received a relatively larger dose. Nevertheless, rhesus No. 302 is the only one of the 4 animals that failed to give any evidence of a reaction (see charts Nos. 3, 4, 5, and 6). Rhesus No. 314 gave a prompt and well-marked reaction. This animal, it will be recalled, was previously used for the unsuccessful attempt at passage from rhesus No. 306. Rhesus No. 315 presented a well-defined but mild reaction, while No. 316 gave indications of a brief abortive reaction that was at its height (40° C.) on the tenth day after inoculation. Both these animals had been subjected to a previous inoculation with typhus fever blood, each having received an intravenous inoculation of 2.5 c. c. of defibrinated blood from a patient (case No. 16–M) in the eleventh day of a sharp attack and when the fever was already falling.

The foregoing test would indicate, therefore, that rhesus No. 302 had developed a resistance to infection with virulent typhus blood as the result of having been bitten by head lice of groups Nos. 3–M and 4–M.

On account of the importance of the question involved it was thought desirable to give rhesus No. 302 a second immunity test. Accordingly on February 1, having been returned to the Hygienic Laboratory, he was given an intravenous injection of 2.5 c. c. of typhus blood (New York virus). At the same time rhesus Nos. 315 and 316 that had served as controls in the previous test were similarly inoculated. Following this inoculation none of these animals gave any appreciable evidence of reaction, although two other monkeys, Nos. 198 and 322, inoculated at the same time, reacted promptly and sharply, testifying to the virulence of the blood used for the test. The result of this test is in harmony with and confirms the result of the previous one and therefore strengthens the conclusion that the resistance of rhesus No. 302 to the immunity test was due to the bites of the head lice to which he was previously subjected.

### *Experiment No. 9.*

Shortly after the lice-feedings on rhesus No. 302 had terminated, a series of feedings with lice of group No. 5–M and group No. 6–M were begun on rhesus No. 304. The lice constituting these groups were obtained from the hair clipped from the scalp of case No. 11–M and of case No. 12–M November 20, 1911, on admission to the typhus ward. One portion of these lice (group No. 5–M) was applied about 24 hours later to rhesus No. 304, 54 of the insects feeding. Thereafter they were applied daily in rapidly diminishing numbers until the morning of November 26, when a single survivor was given its last feed.

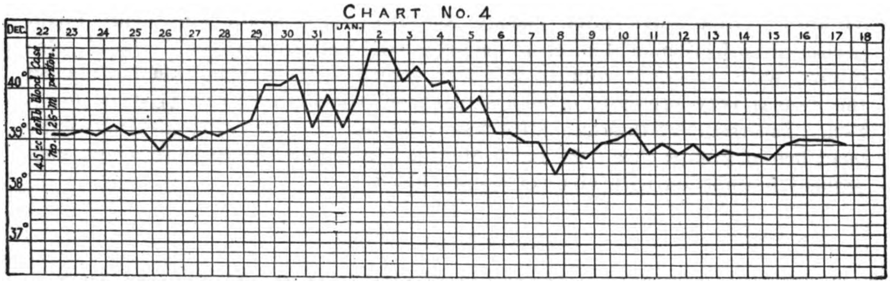

**Chart** No. 4.—Temperature curve of rhesus No. 314 following immunity test; control on rhesus No. 302.

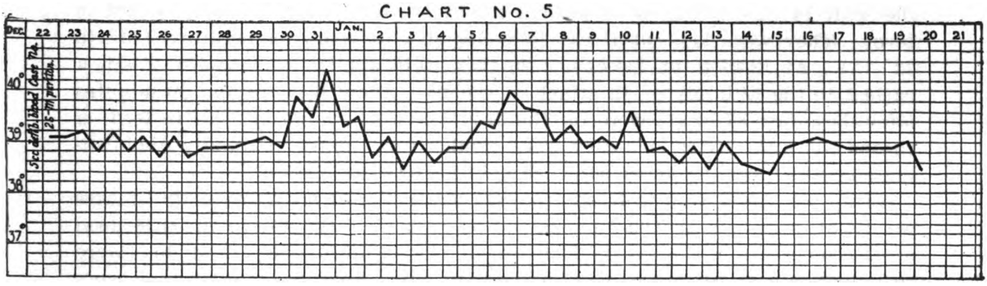

**Chart** No. 5.—Temperature curve of rhesus No. 315 following immunity test; control on rhesus No. 302.

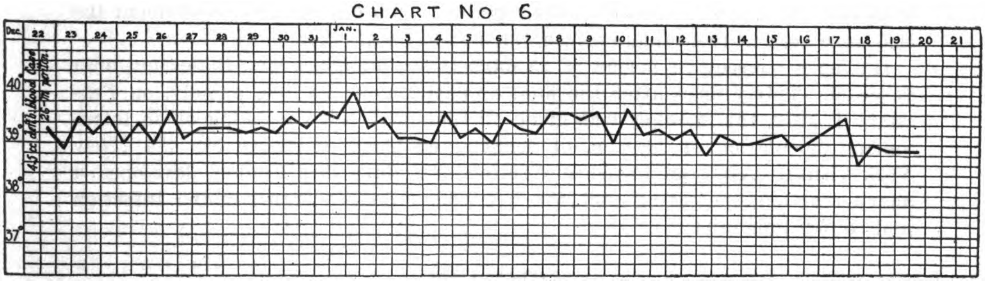

**Chart** No. 6.—Temperature curve of rhesus No. 316 following immunity test; control on rhesus No. 302.

Another portion, 18 in all, of the head lice from cases No. 11–M and No. 12–M (group No. 6–M) were reapplied to case No. 11–M on November 21, or about 24 hours after they were isolated. On the succeeding day they were applied to the monkey and thereafter were applied twice daily in diminishing numbers until the afternoon of November 26, when a single survivor of the group obtained its last feed. Throughout the experiment the lice were kept at room temperature (about 14° to 24° C.). During a period of observation of 32 days following the last exposure to the bites of groups Nos. 5–M and 6–M this animal gave no indication of a reaction. On December 29, or 33 days after the last exposure, this animal was given an intraperitoneal injection of 6 c. c. of defibrinated blood of case No. 35–M diluted with an equal volume of saline solution. Three days later rhesus No. 304 received an additional intraperitoneal injection of 4 c. c. of defibrinated blood from case No. 39–M, also diluted with an equal volume of saline solution. Following this inoculation the animal very promptly developed a sharp reaction of 10 to 11 days' duration. The result of this immunity test indicates that the repeated bites of the head lice of groups Nos. 5–M and 6–M failed to confer any resistance to a subsequent inoculation with virulent typhus blood.

### *Experiment No. 10.*

In this experiment two groups of head lice (Nos. 15–M and 17–M) were repeatedly applied and allowed to feed on rhesus No. 322. Group No. 15–M was isolated in the afternoon of December 19, 1911, from the hair clipped from the scalp of case No. 26–M, a patient in the ninth day of an attack of typhus fever. They were kept until the following day at room temperature (14° to 22° C.). At 12.45 p. m. December 20 they were applied to case No. 27–M, a patient in the 12th day of an attack of typhus. About 3 hours and 20 minutes later these lice, 37 in all, were for the first time applied and allowed to feed on rhesus No. 322. After this they were applied twice daily in rapidly diminishing numbers until December 26, when only 2 survivors remained.

Group No. 17–M was isolated from the hair clipped from the scalp of case No. 28–M, a patient in the 10th day of an attack of typhus, in the afternoon of December 20, 1911. At 11.30 a. m. of the following day these lice were reapplied to case No. 28–M, now in the 11th day. Four hours later they were applied for the first time to rhesus No. 322, 15 feeding on this occasion. Thereafter, these lice were applied twice daily in rapidly diminishing numbers until December 16, when only 2 survivors remained. Throughout the experiment the lice were kept at room temperature (about 14° to 22° C.).

During a subsequent period of 25 days this animal gave no indication of a reaction. Thirty days after the final application of the lice, namely, on February 1, 1912, this monkey was given an immunity test consisting of an intravenous inoculation of 2.5 c. c. of defibrinated blood of rhesus No. 184 (New York virus). After an incubation period of 8 days this monkey developed a sharp reaction, indicating that the lice had failed to transmit the infection and confer immunity.

## SUMMARY.

### I.

(*a*) In one of two attempts to transmit typhus fever (New York virus—Brill's disease) from monkey to monkey by means of the bite of the body louse (*Pediculus vestimenti*) the monkey bitten by the presumably infected lice developed a fever of short duration followed by a relapse and later progressive emaciation, and death, with no macroscopic lesions at necropsy.

(*b*) Two attempts to transmit typhus fever (New York virus) from monkey to monkey by means of subcutaneous injections of suspensions of chloroformized and crushed lice resulted negatively.

(*c*) In one of two attempts to transmit typhus fever (Mexican virus) from man to monkey by means of subcutaneous injection of a saline suspension of crushed body lice, the monkey so inoculated has resisted two subsequent inoculations with virulent typhus blood (New York virus).

The foregoing results are in harmony with and confirm those reported by previous workers.

## II.

In this paper we present the first evidence incriminating any insect other than the body louse as an intermediary in the transmission of typhus fever:

(*a*) In an attempt to transmit typhus fever (Mexican virus) from man to monkey by subcutaneous injection of a saline suspension of crushed head lice (*Pediculus capitis*) the monkey developed a typical febrile reaction with subsequent resistance to an inoculation of virulent typhus (Mexican) blood.

(*b*) In one of three experiments to transmit typhus fever (Mexican) from man to monkey by means of the bite of the head louse (*Pediculus capitis*), the animal bitten by the presumably infected head lice proved resistant to two successive immunity tests with virulent typhus blood.

## CONCLUSIONS.

1. The body louse (*Pediculus vestimenti*) may become infected with typhus. The virus is contained in the body of the infected louse and is transmissible by subcutaneous injection of the crushed insect or by its bite.

2. The head louse (*Pediculus capitis*) may become infected with typhus. The virus is contained in the body of the infected louse and may be transmitted by subcutaneous injection of the crushed insect and, we believe, also by its bite.

## ACKNOWLEDGMENTS.

We desire to renew our acknowledgments of indebtedness to Drs. Nathan E. Brill and Leo Kessel, of New York City, and to Dr. Leon Louria, of Brooklyn, for cases of Brill's disease; to Dr. Eduardo Licéaga, president, and Dr. Octaviano Gonzalez Fabela,

director of the bacteriological laboratory of the Superior Board of Health of Mexico; to Dr. Regino Gonzalez, director of the Hospital General; Dr. German Diaz Lombardo, director of the Hospital Juarez, and Drs. Miguel Otero and Zenon Luna, for laboratory and clinical facilities; for many valuable courtesies to Dr. A. R. Goodman, chief surgeon of the national railways of Mexico, and Drs. Genaro Escalona, Felipe Perez Garza, J. R. Davis, and Señor Salvador Aguirre.

# 6.—STUDIES ON THE VIRUS OF TYPHUS.

By JOSEPH GOLDBERGER, *Passed Assistant Surgeon*, and JOHN F. ANDERSON, *Director Hygienic Laboratory, Public Health Service.*

## DURATION OF THE INFECTIVITY OF THE BLOOD.

The human inoculations by Moczutkowski (1900) and Otero (1908) strongly suggested, and those of Yersin and Vassal (1908) made it highly probable,[2] that the typhus-fever virus resides in the blood. Satisfactory proof of this, however, was not adduced until February, 1910, when Anderson and Goldberger showed that the fever induced in the monkey by an inoculation with human typhus blood, besides being transmissible by passage, conferred a definite immunity to a second inoculation.[3]

Since that time the experimental work of Anderson and Goldberger, of Ricketts and Wilder, of Gaviño and Girard, but more particularly that of Nicolle and Conseil, has developed a solid basis for the conclusion that the virus of typhus may be (or is) present in the blood, at least throughout the febrile period.

Nicolle and Conseil (August, 1910) were the first to try to determine whether the virus is present in the circulating blood before the onset of fever and after its defervescence, and from their experiments they concluded (January, 1911) that typhus blood is virulent, not only throughout the febrile period, but also before the onset of fever and at the beginning of convalescence (second day after defervescence).

In our recent work we repeated Nicolle and Conseil's experiments and have obtained results that cast grave doubt on the validity of Nicolle and Conseil's interpretations.

Before presenting our own work it will be desirable to examine Nicolle and Conseil's experiments somewhat in detail.

In order to determine whether typhus (monkey) blood is still infective after defervescence, Nicolle and Conseil inoculated a bonnet monkey (No. 31) with some blood from a chimpanzee (No. 3) ob-

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[1] Reprinted from the Public Health Reports, vol. 27, No. 22, May 31, 1912, p. 835.

[2] There is some doubt whether Yersin and Vassal were really working with typhus.

[3] Nicolle's proof of the successful inoculation of his chimpanzee was the occurrence of a mild fever 24 days after inoculation and an eruption. Although the injection of 1 c. c. of the chimpanzee's blood was followed, in a bonnet monkey, by fever "with a most characteristic eruption," this was hardly convincing proof that the fever was typhus. It may be remarked in this connection that in his later work Nicolle has ceased to note the occurrence of an eruption.

tained 36 to 48 hours after the return to normal of its temperature. After an incubation of 8 days the temperature of the bonnet monkey rose abruptly, remained up about 24 hours, then dropped, without subsequent hypothermia, but with slight emaciation. This is interpreted by Nicolle and Conseil as a typhus reaction, although when later subjected to an immunity test bonnet monkey 31 presented a well-marked reaction, showing that the fever of some 24 hours' duration, which Nicolle and Conseil interpret as a typhus reaction, had conferred no immunity.

With a view of testing the infectivity of the blood during the incubation of typhus, Nicolle and Conseil inoculated a bonnet monkey (No. 37) with blood from a chimpanzee (No. 4) obtained three days before the beginning of the fever. The result of this inoculation appeared to be absolutely negative, but when subjected to an immunity test 33 days later this animal (bonnet monkey 37) did not react. Whereupon Nicolle and Conseil conclude that the first inoculation, although followed by no symptoms, had conferred on their bonnet 37 a solid immunity.

It is evident that Nicolle and Conseil's conclusions that the blood is infective after defervescence and during the prefebrile period depend on the validity of their interpretations of the two experiments just cited.

The validity of their interpretation of the first of the above experiments is clearly involved in the question of what constitutes a typhus reaction. We defer a full discussion of this question to a later paper. At this time we wish simply to state that we are convinced that in the present state of our knowledge it is not permissible to interpret as a typhus reaction in the monkey a fever that confers no immunity.

In the second of the two experiments cited Nicolle and Conseil base their interpretation on the outcome of the immunity test; as this was negative they conclude that the animal, though it had presented no symptoms, was vaccinated by the injection with the blood obtained from chimpanzee No. 4.

It is obvious that in this case Nicolle and Conseil disregard the possibility of a natural immunity in the monkey. This possibility is not as remote as the early workers, including ourselves, believed. We have met with it several times in our recent work. Instances will be cited in other portions of this paper, although we shall defer a full discussion of this important matter to a later communication. For our present purpose it will be enough to say that in several instances in our recent experience monkeys have resisted one or two inoculations with virulent blood or blood serum and yet have reacted sharply and characteristically to the second or the third.

From the foregoing discussion it is clear that Nicolle and Conseil's conclusions are not justified by the results of their experiments when these are strictly interpreted.

Our own experiments follow:

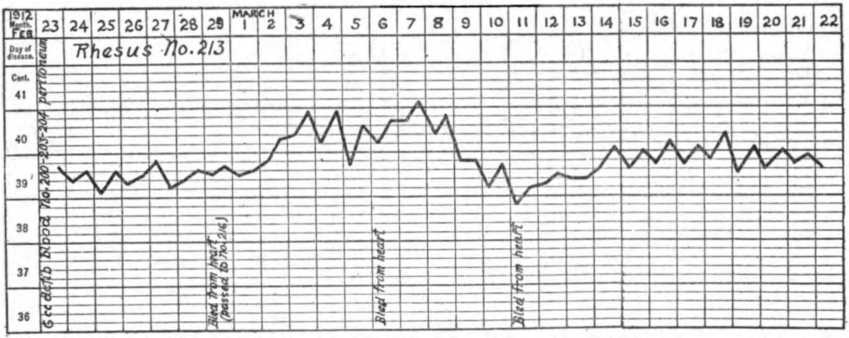
### *Experiment No. 1.*

At 12 m., February 29, about 32 hours before the beginning of a well-marked typhus reaction, we aspirated blood from the heart of rhesus No. 213 (chart 1) and after defibrination injected 8 c. c. of it diluted with 4 c. c. of saline solution into the peritoneal cavity of rhesus No. 216.

*Result:* During a period of observation of 30 days rhesus No. 216 failed to give any evidence of a reaction. At the end of this period he was subjected to an immunity test consisting of an intraperitoneal injection of 3 c. c. of defibrinated blood, obtained from monkeys Nos. 232 and 233, diluted with 2 c. c. of saline solution. To this test rhesus No. 216 reacted promptly and characteristically.

It appears, therefore, that monkey No. 216 was neither infected nor vaccinated by the inoculation with the blood of No. 213. This result would seem to justify the interpretation that the blood obtained from rhesus No. 213 about 32 hours before the beginning of fever was not virulent. This interpretation, though probably correct, is not permissible, in view of our experience that the normal monkey may resist one or two or more inoculations with virulent blood.

CHART No. 1



Temperature curve of rhesus No. 213.

### *Experiment No. 2.*

At 3.50 p. m., March 11, 1912—that is, at least 24 to 32 hours after the temperature of rhesus No. 213 reached normal—we again drew blood from this animal's heart and after defibrination, 9 c. c. of it diluted with 3 c. c. of saline solution was injected intraperitoneally into rhesus No. 227.

*Result:* After an incubation period of about 24 days this monkey developed a mild fever of about 7 days' duration, which is sufficiently definite to justify us in considering it a typhus reaction (chart 2).

The mild reaction obtained in rhesus No. 227 following inoculation with blood obtained at least 24 to 32 hours after the temperature of rhesus No. 213 reached normal justifies the interpretation that this blood was infective.

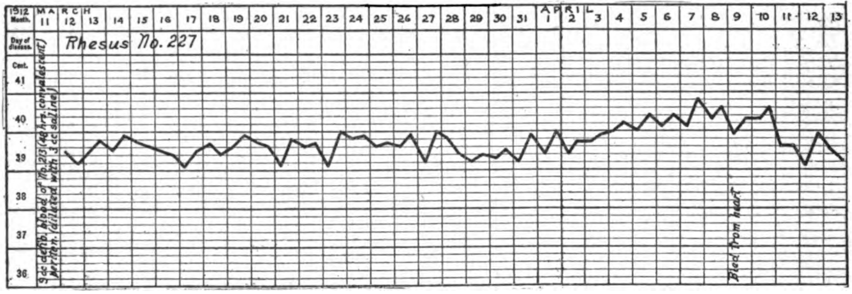
Conclusions.—From the foregoing discussion and the results of our experiments the following conclusions seem to us permissible:

1. The blood of the monkey infected with typhus may be virulent in the prefebrile stage, but no satisfactory evidence of that fact has as yet been adduced.
2. The blood of the monkey may still be virulent 24 to 32 hours after the return of the temperature to normal.

## THE SEAT OF THE VIRUS OF TYPHUS.

The demonstration of the presence of the typhus virus in the circulating blood naturally leads one to inquire as to the element of the blood in which it resides. Nicolle and Jaeggy (April, 1910) were the first to consider this problem. In their hematologic study of typhus they describe a peculiar necrosis of the polynuclears which they consider significant as regards the seat of the unknown infectious agent, an agent which they therefore think is probably an intraleucocytic parasite.

CHART No. 2



Temperature curve of rhesus No. 227. Mild febrile reaction following inoculation with blood obtained in beginning of convalescence.

Somewhat later Nicolle, Conor, and Conseil (October, 1910) point out certain apparent differences between the infectivity of typhus blood serum obtained after centrifugation of defibrinated blood and that obtained after clotting. These differences appear to them to support the intracellular hypothesis first suggested by Nicolle and Jaeggy.

In a later paper (January, 1911) Nicolle, Conor, and Conseil write that if the hypothesis of the intracellular character of the virus be accepted—

> the discordant results of inoculation with serum obtained by centrifugation of defibrinated blood and that obtained after coagulation are easily explained. The former is always virulent, because no matter how rapid and perfect the centrifugation may have been, it is bound to contain cellular débris and organisms liberated by the destruction of cells in the act of defibrination. The serum obtained after coagulation is, theoretically, deprived of all virulence for the monkey because it is freed of most of the liberated organisms and almost completely of the suspended cells by the formation and retraction of the clot.

They add, however, that—

> the absence of virulence of the serum obtained after coagulation may not be absolute. When the serum does not separate well from the clot and when for that reason it contains a certain number of cells or when the amount inoculated is large the infection of the monkey may be realized.

In still a later paper (September, 1911) Nicolle, Conor, and Conseil report experiments especially designed to test their hypothesis. They consider that the results of these experiments support the theory of intraleucocytic localization.

On account of the great importance of the question involved it seems desirable to examine these experiments in detail. They are as follows:

### A. COMPARATIVE VIRULENCE OF THE VARIOUS ELEMENTS OF THE BLOOD SEPARATED BY CENTRIFUGATION.

#### *Experiment 1.*

The blood used was drawn by venepuncture of patient 120 in the seventh day of a severe typhus, to which was added some sodium citrate, centrifugated,[1] etc. The manipulation of separation and lavage of the different elements of the blood took three-quarters of an hour.

Seven bonnet monkeys were inoculated intraperitoneally as follows: A fresh control and two immunes each with 5 c. c. of the fresh blood; monkey A with the same dose of citrated blood; monkey B with the same dose of citrated plasma; monkey C with 2.5 c. c. of red corpuscles; monkey D with 1 c. mm. of the leucocyte layer.

The results were as follows: Fresh control (fresh blood), incubation 16 days, typhus of median severity of 12 days' duration (the temperature was over 40° during 6 days); immunes (fresh blood), negative. A (citrated blood), incubation 12 days, typhus of median severity of 9 days' duration (the temperature was over 40° during 9 days); B (citrated plasma), incubation 8 days, duration 9 days (6 days above 40°); C (red corpuscles), incubation 13 days, typhus abortive of 5 days (3 days at 40°, of which only one above); D (white corpuscles), incubation 6 days, typhus grave of 9 days (9 days above 40°).

#### *Experiment No. 2.*

Performed under the same conditions as No. 1 with the blood of patient 69 in the thirteenth day of a severe attack. Separation of the various elements of the blood took 30 minutes and was more perfect than in the first experiment. Three bonnet monkeys were inoculated intraperitoneally as follows: Monkey E with 5 c. c. of citrated plasma, F with the same dose of red corpuscles, G with about 1 c. mm. of leucocytes.

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[1] Just as we were about to go to press Nicolle, Conor, and Conseil's paper (Apr. 25, 1912) came to our hands, from which we cite the following additional details of this experiment: "After centrifugation (lasting 12 minutes) we at once removed several cubic centimeters of plasma from the upper portion of the tubes. The leucocyte layer was carefully removed and put in saline solution; several cubic centimeters of red corpuscles were then removed from the deepest portion of the tubes and placed in some of the same solution. These manipulations required 22 minutes. After that the white and the red globules suspended in saline solution were submitted to a second centrifugation of 10 minutes' duration. This done, it required 6 minutes more to separate the cells from the liquid in which they were washed."

The results were as follows: E (citrated plasma), incubation 13 days, mild typhus of 7 days' duration (3 days above 40°); F (red corpuscles), negative; G (white corpuscles), incubation 7 days, severe typhus of 11 days (temperature above 40° during 9 days). Tested 67 days after the first inoculation by the intraperitoneal injection of 5 c. c. of blood from a monkey in the fifth day of an experimental attack; E and G have not reacted, F developed a severe typhus of 8 days after an incubation of 11 days.

### B. NONVIRULENCE OF BLOOD SERUM FREED OF ITS CELLULAR ELEMENTS BY CENTRIFUGATION.

Serum obtained after clotting is, theoretically at least, free of cells. These remain entangled in the clot as it retracts; prolonged centrifugation frees it of such formed elements as it may contain. It is easy therefore to obtain a serum free of all white corpuscles. If our hypothesis as to the localization of the typhus virus in these cells is correct, such a liquid will be inoffensive. The experiment performed on man shows that it is so.

One of us received intravenously 1 c. c. of blood serum from a monkey in the third day of typhus seven hours after the blood was drawn; result negative. A previous experiment had shown us that the blood serum has no microbicidal action in vitro on the typhus virus.

### C. NONVIRULENCE OF THE CEREBROSPINAL FLUID.

This liquid, destitute of all cellular elements, possesses no virulence.

"A bonnet monkey inoculated intraperitoneally with 15 c. c. of cerebrospinal fluid of patient 28 in the ninth day of a severe attack has presented no symptoms; tested 14 days later by an intraperitoneal inoculation of 5 c. c. of blood of a monkey in the fifth day, this monkey developed a fever of 7 days' duration after an incubation period of 12 days."

From these experiments Nicolle, Conor, and Conseil draw the following conclusions:

1. Of the various elements of the blood separated by centrifugation the leucocytes are the most virulent; a minimal dose of these cells promptly determines in the monkey a grave infection.
2. The plasma, less active, seems to owe its virulence only to the leucocytes or leucocytic débris, of which it is difficult to free the plasma completely; the washed red cells are not virulent.
3. Centrifugated blood serum is inoffensive for man and a humor destitute of cells; the cerebrospinal fluid is equally inactive.

Before discussing the foregoing experiments and conclusions we desire to detail some pertinent experiments of our own.

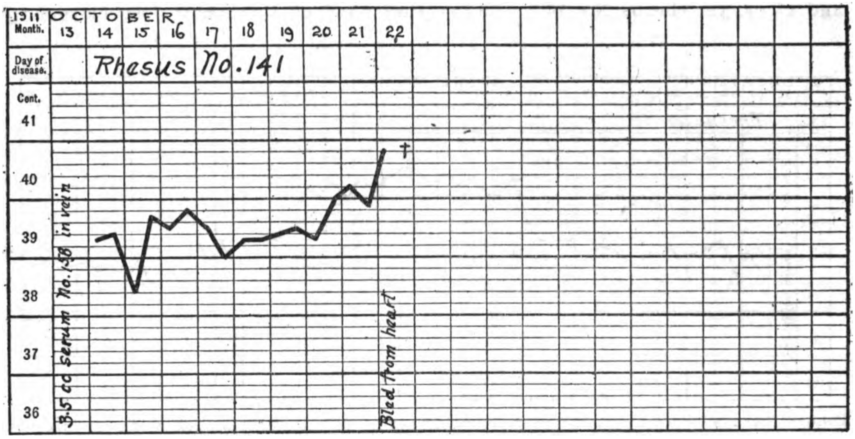
### *Experiment No. 3.*

On October 13, 1911, some defibrinated blood from rhesus No. 158, then in the first day of a typhus reaction, was centrifugated for about 30 minutes, after which the clear supernatant serum was pipetted off. With some of this serum two rhesus monkeys were inoculated. No. 141 received 3.5 c. c. intravenously; No. 142 received 0.5 c. c subcutaneously and 4 c. c. intraperitoneally.

*Result:* Rhesus No. 141 developed a sharp reaction 7 days after the inoculation (chart 3); but during a period of observation of 29 days rhesus No. 142 gave no evidence of a reaction. At the end of this period No. 142 was subjected to an immunity test consisting of an intravenous injection of 2.5 c. c. of defibrinated blood of rhesus No. 163, to which he responded promptly and sharply. (Chart 4.)

Although one of the two animals inoculated (with the larger dose) did not respond and was not vaccinated, it is clear that the blood serum of rhesus No. 158 was infective.

CHART NO. 3

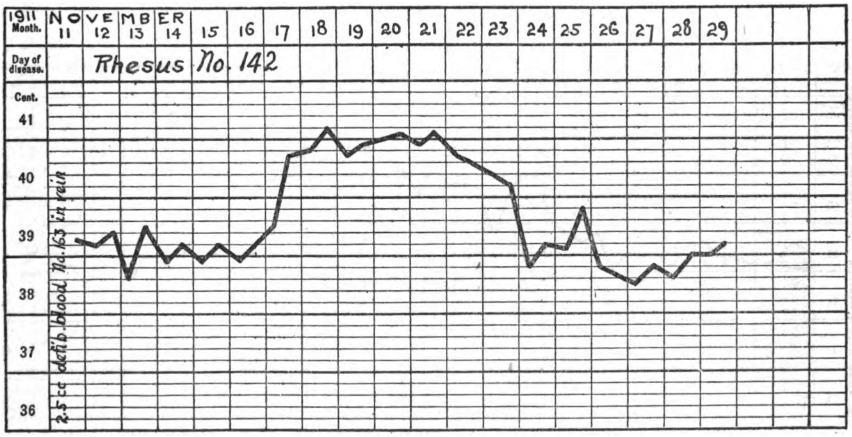


Temperature curve of rhesus No. 141, showing first three days of typhus reaction following inoculation with blood serum.

*Experiment No. 4.*

On October 22, 1911, blood was drawn from rhesus No. 141, then in the third day of a marked attack of experimental typhus (chart 3), and allowed to clot in a tube. After 3 hours, in order to separate the serum more thoroughly from the clot, the tube was centrifugated for about 15 minutes. After drawing off the clear straw-tinted serum a portion of it was used for the inoculation of two monkeys. No. 147 was given 4.5 c. c. and No. 148, 3 c. c., both intravenously. The time that elapsed between drawing the blood and inoculating was about 3 hours and 20 minutes.

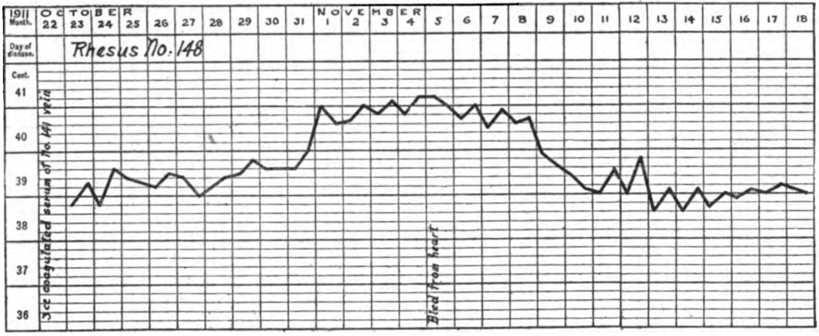
CHART NO. 4



Temperature curve of rhesus No. 142, showing reaction to immunity test.

***Result:*** During a period of observation of 29 days rhesus No. 147 gave no evidence of a reaction. Rhesus No. 148, however, after an incubation period of about nine days, developed a severe typhus, as shown by marked fever (chart 5) and successful passage to another monkey. It should be noted that, although rhesus No. 147 and rhesus No. 148 were of substantially the same size and vigor, yet rhesus No. 147—the one that received the larger dose (larger by 50 per cent)—failed to react, and when subjected to an immunity test, consisting of a subcutaneous injection of about 0.5 c. c., and of an intravenous injection of 2 c. c., of defibrinated blood of rhesus No. 166, then in the third day of an experimental typhus, rhesus No. 147 reacted promptly and sharply (chart 6).

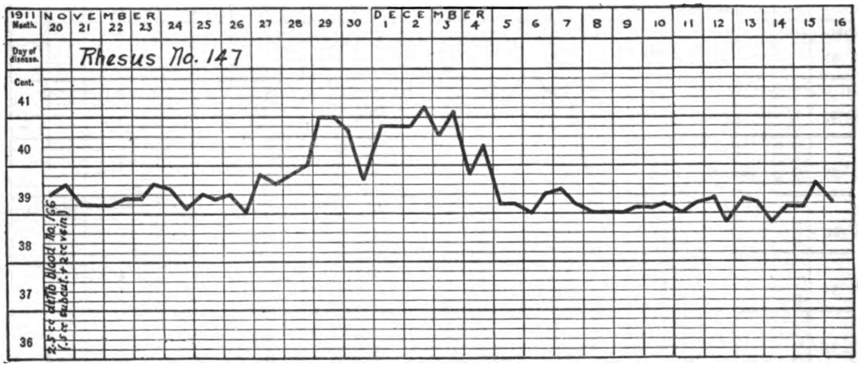
CHART No. 5.



Temperature curve of rhesus No. 148, showing severe febrile reaction following inoculation with blood serum.

Although one of the two animals inoculated (with much the larger dose) did not become infected nor immunized, it is evident that the blood serum of rhesus No. 141, obtained after clotting and centrifugation, was infective.

CHART No. 6



Temperature curve of rhesus No. 147, showing reaction to immunity test.

*Experiment No. 5.*

On March 6, 1912, blood was aspirated from rhesus No. 210 and No. 213. The former was in the seventh and the latter in the fifth day of an experimental typhus. The blood was allowed to clot in tubes and, as in Experiment No. 4, after standing three hours it was centrifugated in order better to separate the serum from the clot. The serum was then pipetted off and mixed. Of this serum, 6 c. c. were intraperitoneally injected into rhesus No. 221.

*Result:* During a period of observation of 24 days following this inoculation monkey No. 221 manifested no recognizable symptoms of a reaction. He was therefore reinoculated March 30 in order to test his immunity; for this purpose he was given an intraperitoneal injection of 3 c. c. of virulent defibrinated blood (rhesus Nos. 232 and 233). Twenty-one days later, April 20, not having in the meantime given any indications of a reaction, he was again given an intraperitoneal injection of 3 c. c. of virulent defibrinated blood (rhesus No. 225). So far this monkey has given no recognizable evidence of a reaction following this inoculation.

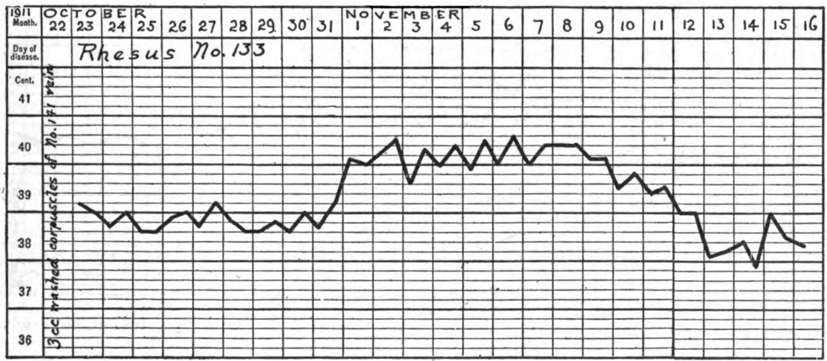
The results of the repeated immunity tests of rhesus No. 221 would seem to indicate that this animal was vaccinated by the first inoculation with 6 c. c. of serum; we think it more probable, however, that this is an instance of natural immunity in this monkey.

### *Experiment No. 6.*

On October 27, 1911, some blood drawn from rhesus No. 141 (chart 3) was defibrinated and 3 c. c. of it tubed and centrifugated. After a centrifugation of about 15 minutes the serum was pipetted off and replaced by 5 c. c. of saline solution in which the corpuscles were thoroughly stirred up. The tube was then again put in the centrifuge for 15 minutes, after which it was taken out and the supernatant liquid pipetted off and replaced by 5 c. c. of fresh saline solution. This maneuver was twice repeated. In other words, the corpuscles were washed three times in an excess of saline solution. After the third washing the corpuscles, in fresh saline solution, representing originally 3 c. c. of defibrinated blood, were injected intravenously into rhesus No. 133.

*Result:* After an incubation period of about 10 days this monkey developed a marked typhus reaction (chart No. 7).

CHART No. 7



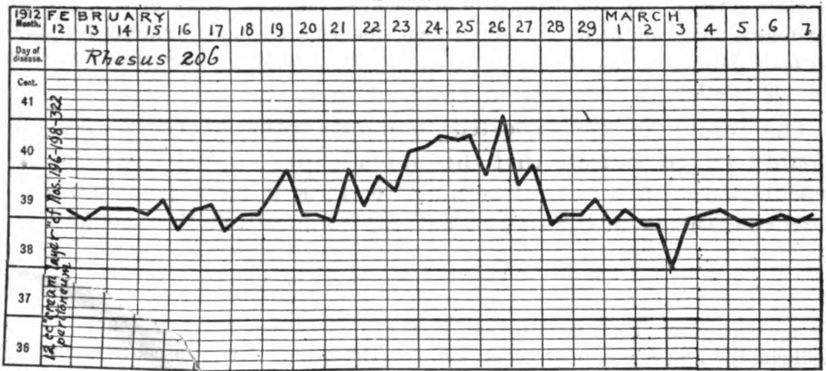
Temperature curve of rhesus No. 133, showing reaction following inoculation with washed corpuscles.

Washing the blood corpuscles three times in an excess of saline solution had, therefore, not deprived them of the power to infect.

### *Experiment No. 7.*

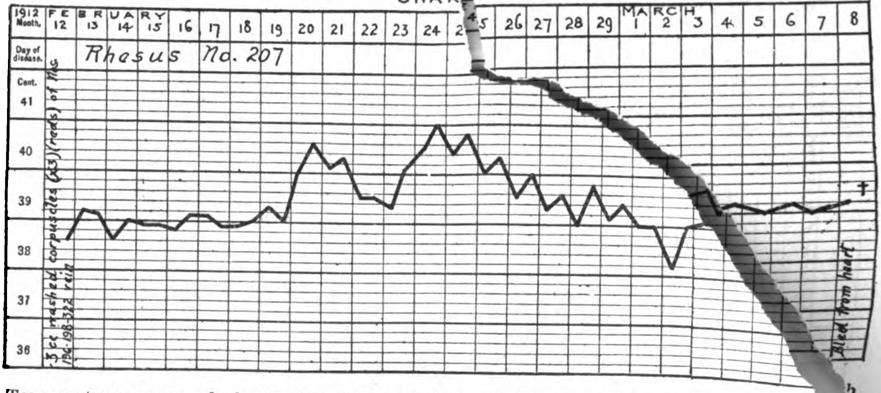
**On February 12, 1912, we aspirated blood from three monkeys—Nos. 196, 198, and 322—that were in the fourth, fifth, and sixth days, respectively, of experimental typhus. The blood was defibrinated and mixed. The corpuscles of 12 c. c. of this mixed defibrinated blood were washed three times, as in experiment No. 6.** After the third washing the supernatant liquid was drawn off and discarded; then the surface layer ("cream" or leucocyte layer) of corpuscles was pipetted off and injected intraperitoneally into rhesus No. 206. Of the corpuscles remaining 3 c. c. were intraperitoneally injected into rhesus No. 207.

CHART No. 8



Temperature curve of rhesus No. 206, showing reaction following an inoculation with leucocyte layer of washed corpuscles.

CHART No. 9



Temperature curve of rhesus No. 207, showing reaction following an inoculation with washed "red" corpuscles.

*Result:* Both monkeys developed well-marked reactions after an incubation period of seven days in No. 206 and eight days in No. 207. Although the reaction in neither was severe, that in No. 207 was perhaps the somewhat better marked (charts 8 and 9).

Both the "cream" or leucocyte layer and the red[1] corpuscles of washed blood were, therefore, infective.

Discussion.—Nicolle, Conor, and Conseil's interpretations are evidently based on the assumption that the severity of the reaction is a direct index of the virulence of the infecting dose.

If this assumption were permissible it would be difficult to say from the character of the reaction noted by us in rhesus No. 206 and No. 207 (charts 8 and 9) which was the more virulent, the "cream" layer given to No. 206 or the "reds" given to No. 207. As a matter of fact, there does not appear to be any necessary relation between dosage and severity of reaction.

Ricketts and Wilder (Feb. 5, 1910) and Anderson and Goldberger (Feb. 18, 1910) almost simultaneously record observations to this effect over two years ago. We have an extreme and a very pertinent illustration of this in the result above detailed of our experiment No. 4, in which, of two animals of equal size and vigor inoculated with typhus blood serum, the monkey that received the smaller dose presented a severe reaction, while the monkey that received the larger dose (by 50 per cent) failed altogether to react, although a month later he reacted promptly and sharply to an immunity test with a relatively small amount of defibrinated blood. But waiving this objection and accepting their interpretation that "of the various elements of the blood separated by centrifugation the leucocytes are the most virulent," "the plasma less active," "and the washed corpuscles not virulent" ("not virulent" in the sense, it is presumed, of "low virulence," for in one of their experiments under "A" the "reds," which necessarily entangled some leucocytes, were infective, as they were also in our experiment No. 7), it does not seem to us necessarily to follow that because the degree of virulence of the various elements of the blood thus separated appears to run parallel[2] with their (incorrectly) assumed richness in leucocytes that the virus is localized in the leucocytes or has any relation to the leucocytes other than that of possessing, possibly, a specific gravity approximating, or identical with, that of these cells.

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[1] Although centrifugation separates the white and the red corpuscles, this separation is never complete. A considerable number of leucocytes are always carried down by the reds from which they can not be completely removed, even by repeated washings and repeated removal of the "cream" layer. So that when we speak of the "reds" it must always be understood that a not inconsiderable number of whites are included. This is well illustrated by the following experiment, kindly made for us by our colleague, Dr. Leake: Some typhus monkey blood was citrated and then centrifugated for one hour. After this the serum and the cream layers were pipetted off and the remaining "reds" washed three times. After each washing the "cream" layer that formed was discarded. Leucocyte counts were made as follows: (1) Citrated blood before centrifugation, 7,000 per c. mm.; (2) plasma after centrifugation of 1 hour, less than 10 per c. mm.; (3) "cream" layer after centrifugation of 1 hour, 79,000 per c. mm.; (4) "reds" after centrifugation 1 hour and after removing the "cream," 14,000 per c. mm.; (5) "reds" after washing three times and each time discarding the "cream" layer, 9,000 per c. mm. After an hour's centrifugation, therefore, the plasma of citrated blood was not freed of leucocytes and the number of leucocytes among the "reds" was relatively increased.

[2] As a matter of fact, there is no evidence to show that it really does, for the leucocyte content of the various "elements" of the blood separated by centrifugation is in the following order: "Cream," "reds," plasma (see note, Experiment No. 7).

In other words, having due regard for the mechanics of centrifugation, one might reasonably expect that the distribution in the different layers of centrifugated blood of an extracellular parasite of approximately the same specific gravity as that of the leucocytes would be similar to, or identical with, that of the latter. This is well illustrated by the following experiment: Some blood was drawn into sodium citrate solution from the heart of a guinea pig infected with anthrax. A measured amount of this was plated in agar. The remaining citrated blood was then centrifugated for one hour, after which measured amounts of the different layers, plasma, "cream," and "reds," uniform with that of the whole blood, were plated. After incubating 18 hours at 37° the plates were examined. The dilutions not having been sufficient to permit counting the colonies on some of the plates, estimates of their number were made and then certain relative values given to each, with the following result: "Whole blood," 100; "plasma," 1; "cream," 10,000; "reds," 10. Here we have an enormous excess of organisms in the leucocyte layer and a great reduction in the plasma and in the "reds." The reason for the high virulence of the leucocytic layer, the lessened activity of the plasma, and the "nonvirulence"[1] of the washed red corpuscles is therefore obvious.

If now we bear in mind that the severity of the reaction does not necessarily correspond to the degree of virulence (dosage), even though it may do so under certain circumstances, then we believe we have accurately reproduced in this experiment with anthrax blood the results obtained by Nicolle, Conor, and Conseil after centrifugation of citrated typhus blood.

In view of the foregoing it is easy to conceive that typhus blood serum may by sufficient centrifugation be made noninfective even to so (supposedly) sensitive a subject as man. On account of the variable susceptibility of the monkey, very little significance can be attached to the negative result of the single inoculation with cerebrospinal fluid. It does not prove that this fluid is not infective, although it is in harmony with the result of a human experiment made by Otero in 1908. But even if it did, to have the significance that Nicolle, Conor, and Conseil seem to attach to it, it would be necessary to show, first, that the cerebrospinal fluid of typhus is quite free of leucocytes, and second, that in infections due to an extracellular parasite this fluid is invariably virulent.

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[1] Nicolle, Conor, and Conseil say: "Des divers éléments du sang, séparés par centrifugation, les globules blancs sont en effet les plus virulents; une dose minime de ces cellules détermine chez le singe une infection rapide et grave; le plasma, moins actif, semble ne devoir sa virulence qu'aux leucocytes ou débris leucocytaires dont il est malaisé de le débarrasser complètement; les globules rouges lavés n'ont pas de virulence."

We presume that by "n'ont pas de virulence"—have no virulence—they mean "low virulence," for in one of their experiments under "A" the "reds" were infective. A literal interpretation of this phrase would make their argument in favor of an intraleucocytic localization absurd, for the "reds" necessarily entangle a not inconsiderable number of leucocytes as they are precipitated by the centrifuge.

Conclusions.—From the foregoing discussion we believe it permissible to conclude: (1) That the evidence adduced by Nicolle, Conor, and Conseil does not especially favor their hypothesis of an intraleucocytic localization of the virus of typhus—on the contrary, the infectivity of centrifugated blood serum, obtained after clotting, with its low leucocyte content would be in favor of a parasite free in the circulating plasma of the blood; (2) that the blood serum of virulent typhus blood is constantly infective, whether obtained from defibrinated blood or after clotting, instances of its apparent avirulence being explicable by a natural resistance of the monkey; (3) that it may perhaps be possible to deprive typhus blood serum (obtained after clotting) of its virulence by prolonged centrifugation, but that this does not necessarily indicate an intraleucocytic localization of the virus; and (4) that repeated washings of the blood corpuscles do not deprive them of their infectivity, a fact explicable by the physical phenomena involved in centrifugation.

## FILTERABILITY.

### VIRUS IN THE BLOOD.

The question of the filterability of the virus as it exists in typhus blood has been studied by several groups of workers. Ricketts and Wilder were the first to come forward with an answer to this question. On February 5, 1910, they reported a filtration experiment. In this a monkey inoculated with filtered (Berkefeld candle) blood serum (from defibrinated blood) failed to react. Almost simultaneously Goldberger and Anderson (Feb. 18, 1910) reported a similar experiment with a like result. In a paper published some weeks later (Apr. 23, 1910) Ricketts and Wilder report apparently the same result from a second experiment.

In October, 1910, Nicolle, Conor, and Conseil reported two series of experiments with filtered serum (from clotted blood) on monkeys and a third on man. In the first, one of the two monkeys that had received the filtered serum gave no indications of a reaction; the other (bonnet 47) presented an elevation of temperature of 0.5 degree between the sixteenth and eighteenth days,[1] so that the result was doubtful. When later subjected to an immunity test bonnet 47 proved refractory, from which they conclude that the subcutaneous inoculation of filtered typhus serum produced in this monkey a feeble thermic reaction (doubtful), which, however, conferred an absolute immunity to a subsequent immunity test.

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[1] In their summary January, 1911, this is stated as "0.5° to 0.8° between the fifteenth and twenty-first day."

In the second series of their experiments neither the monkey that received the unfiltered serum nor the three monkeys that received the big doses of the filtered serum presented the slightest indications of a reaction.

In a third experiment one of the authors submitted himself to a subcutaneous injection of 0.25 c. c. of filtered typhus serum without developing any symptoms.

After reviewing the results of filtration recorded by Ricketts and Wilder and by Anderson and Goldberger, Nicolle, Conor, and Conseil (January, 1911) conclude " that the serum obtained by centrifugation of defibrinated blood is always inactive after filtration; that the serum obtained after coagulation is generally also inactive after filtration, but not constantly so; and that the only hypothesis that permits of an explanation of these facts is that under ordinary conditions the unknown microbe of typhus is present in the filtrate in numbers too small to cause infection or immunization of the inoculated animal. This microbe is therefore filterable and probably intraleucocytic." It should here be noted that this was written before the result of the immunity test of the monkey used in the second of Ricketts and Wilder's filtration experiments was published. This result appeared in a paper published by Wilder in July, 1911, and showed that as in one of Nicolle, Conor, and Conseil's experiments the monkey that had received the injection of filtered (defibrinated blood) serum was resistant to the immunity test.

In September, 1911, Nicolle, Conor, and Conseil reported still another filtration experiment. Believing that the virus is intraleucocytic they thought " that it would perhaps be possible to obtain, by the artificial disintegration of a large number of these cells, enough free organisms so that their filtered emulsion would infect the monkey.' This, however, did not prove to be the case and they conclude that " in this instance, again, the number of organisms that passed the filter was undoubtedly insufficient," so that their " previous positive filtration experiment remains unique." They are evidently still ignorant of Ricketts and Wilder's " positive " (?) result.

In November, 1911, Gaviño and Girard published some studies in typhus, in which they report an absolutely negative result following inoculation of a monkey with filtered (through a Berkefeld candle) peritoneal exudate rich in erythrocytes and leucocytes, particularly polynuclears. This was obtained from the peritoneal cavity of a monkey at the height of an experimental typhus three hours after injecting some peptonized broth.

Summarizing the foregoing, we have eight attempts, so far recorded, to pass the virus of typhus through a Berkefeld filter. Of these, six were negative; in one of the other two (Wilder, 1911) the monkey that had been inoculated with the filtrate, without giving any evidence of a reaction, was later found to be resistant to an immunity test with virulent blood; in the other (Nicolle, Conor, and Conseil, January, 1911), one of a pair of monkeys inoculated with some of the filtrate is described as having presented a doubtful reaction, and later was found resistant to an inoculation with virulent blood.

Before discussing these results and their significance, we desire to record some new experiments of our own.

### *Experiment No. 8.*

On October 13, 1911, some blood was drawn from rhesus No. 158, then in the first day of a severe experimental typhus. After defibrination this blood was centrifugated for about half an hour, after which the supernatant serum was pipetted off. A portion of the serum was diluted with three volumes of saline solution and then passed through a Berkefeld candle, after which the following inoculations were made: Of the unfiltered serum rhesus No. 141 was given 3.5 c. c. intravenously and rhesus No. 142, 4 c. c. intraperitoneally and 0.5 c. c. subcutaneously. Of the diluted filtered serum rhesus No. 143 was given 8.5 c. c. (representing 2.12 c. c. of the original) intravenously and 6 c. c. (representing 1.5 c. c. of the original) intraperitoneally; rhesus No. 144 was given 9.5 c. c. (representing 2.4 c. c. of the original) intravenously and 5 c. c. (representing 1.25 c. c. of the original) intraperitoneally.

*Result:* Rhesus No. 141 (unfiltered serum), after an incubation period of 7 days, developed a sharp reaction (chart 3), on the third day of which he was sacrificed in the act of aspirating blood for passage. Rhesus No. 142 (unfiltered serum), 143 (filtrate), 144 (filtrate), during a period of observation of 29 days gave no evidence of a reaction. At the end of this period these three monkeys were subjected to an immunity test, each receiving intravenously 2.5 c. c. of defibrinated blood of rhesus No. 163, then in the second day of a marked experimental typhus. All three animals responded promptly and sharply to this test (chart 4).

It appears, therefore, that of the two monkeys (Nos. 141 and 142) that were inoculated with virulent typhus serum obtained after a half hour's centrifugation of virulent defibrinated blood (virulent for two monkeys in a dose of 3.5 c. c. each, intravenously) one (the one receiving the larger dose) failed to react without being refractory to the immunity test and that neither of the two monkeys inoculated with the filtered serum was either infected thereby or had any resistance conferred upon it.

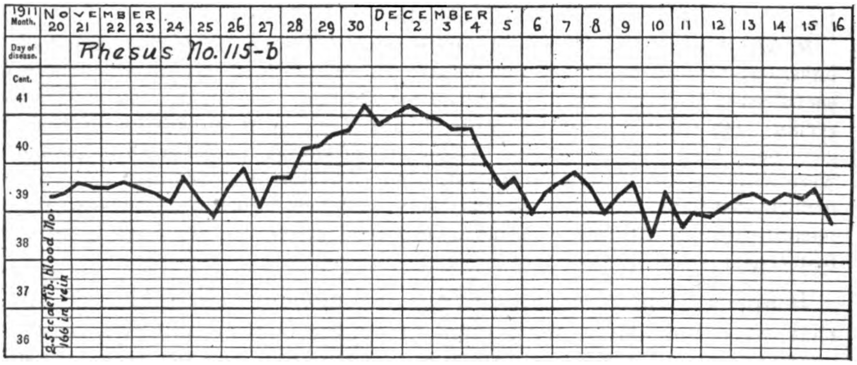
### *Experiment No. 9.*

On October 22, 1911, blood was drawn from the carotid of rhesus No. 141, at this time in the third day of a marked attack of experimental typhus, and allowed to clot in a tube. At the end of 3 hours the tube was centrifugated for about 15 minutes in order to separate the serum more thoroughly from the clot. After drawing off the clear straw-tinted serum a portion was diluted with 3 volumes of saline solution and passed through a Berkefeld filter, after which the following inoculations were made: Of the unfiltered serum, rhesus No. 147 was given 4.5 c. c. and No. 148, 3 c. c., both intravenously. Of the dilute filtered serum rhesus Nos. 115*a* and 115*b* were each given 10 c. c. (representing 2.5 c. c. of undiluted serum) intravenously and 2 c. c. (representing 0.5 c. c. of undiluted serum) intraperitoneally.

*Result:* As already stated in a previous connection, rhesus No. 148 (3 c. c. serum) developed a severe typhus reaction after an incubation period of about

9 days (chart 5). Rhesus No. 147 (4.5 c. c. serum), No. 115*a* (3 c. c. filtered serum), No. 115*b* (3 c. c. filtered serum) during a period of observation of 29 days following the inoculation, presented no recognizable evidence of a reaction. At the end of this period of observation monkeys Nos. 147, 115*a* and 115*b* were subjected to an immunity test consisting of an inoculation of defibrinated blood of rhesus No. 166 then in the third day of a marked experimental typhus, No. 147 receiving 2 c. c. intravenously and 0.5 c. c. subcutaneously, No. 115*a* 2.5 c. c. subcutaneously, and No. 115*b* 2.5 c. c. intravenously. Rhesus Nos. 147 and 115*b* reacted promptly and sharply to this test (charts 6 and 10). No. 115*a*, however, gave no evidence of a reaction during a period of 33 days following this test. Sixty-two days after the inoculation with the filtered serum rhesus No. 115*a* was given a second immunity test consisting of an inoculation, part intravenous and part subcutaneous, of 2.5 c. c. of virulent defibrinated blood of rhesus No. 176, then in the second day of a marked attack. Twenty-eight days later, or 90 days after the inoculation with filtered serum, having failed to react to the second test, this animal was subjected to a third immunity test consisting of an intravenous inoculation of 3.5 c. c. of mixed defibrinated blood from rhesus Nos. 308 and 309, both of which were then in the second day of marked experimental attacks.

CHART NO. 10



Temperature curve of rhesus No. 115*b*, showing reaction following immunity test.

Following this inoculation rhesus No. 115*a*, after an incubation period of eight days, developed a well-marked typhus reaction (chart 11).

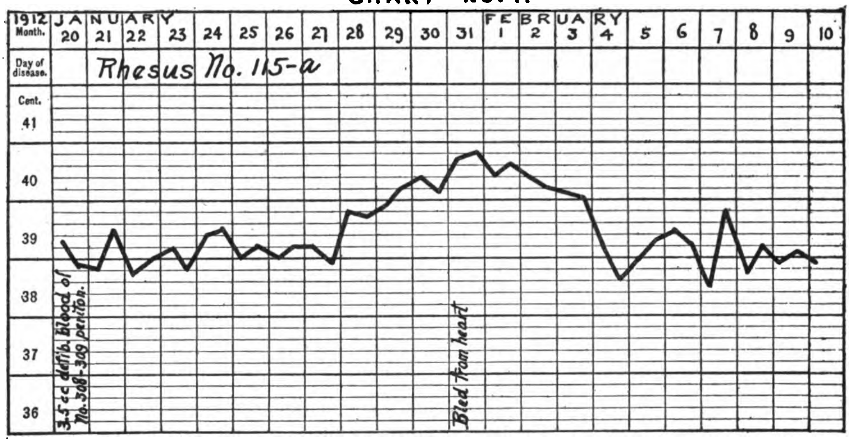
It would appear therefore (1) that of the two animals that were inoculated with the unfiltered serum obtained after coagulation and centrifugation of virulent blood, one (the one receiving the larger dose) failed to react without, however, being resistant to the subsequent immunity test and (2) that neither of the two monkeys inoculated with the filtered serum was infected nor were they vaccinated by the inoculation, although the failure of one of them (115*a*) to react to two successive immunity tests might have suggested that he had been vaccinated had he not been given a third test, to which he responded promptly and definitely.

### *Experiment No. 10.*

On January 20, 1912, some blood was drawn into a tube from the carotid of rhesus No. 309, which at this time was in the second day of a sharp attack of the experimental disease. This blood was allowed to clot at room temperature.

At the end of two and one-half hours it was centrifugated, after which the perfectly clear serum was pipetted off and a portion diluted with 2 volumes of saline solution. This diluted serum was then passed through a Berkefeld candle, after which the following inoculations were made: Rhesus No. 194 was given 18 c. c. of the filtrate (representing 6 c. c. of serum) intraperitoneally; rhesus No. 195 was given 6 c. c. of unfiltered serum, likewise intraperitoneally.

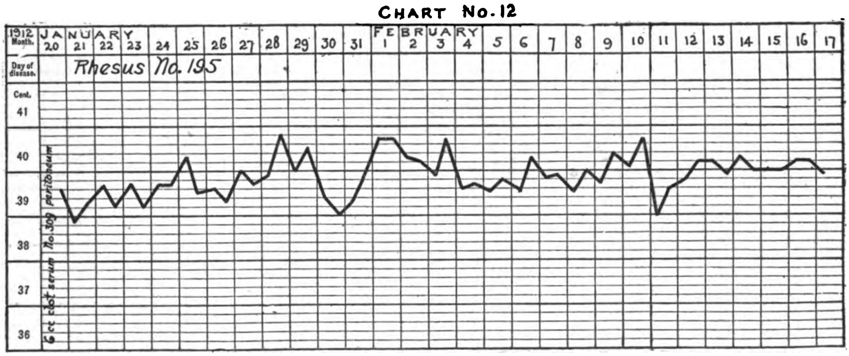
CHART No. 11



Temperature curve of rhesus No. 115*a*, showing reaction following third immunity test.

*Result:* Rhesus No. 195 developed what was probably a typhus reaction after an incubation period of 8 days (chart 12). Some doubt, however, is cast upon this interpretation by the fact that this monkey's normal temperature was somewhat high, due, probably, to a coexisting tubercular infection that later was found to be present. Rhesus No. 194 presented no indications of a reaction during a period of observation of 34 days. At the end of this time he was subjected to an immunity test consisting of an intraperitoneal injection of 6 c. c. of virulent defibrinated blood without eliciting any evidence of a reaction. This would suggest that this monkey had been made refractory by the inoculation with the filtrate, just as appeared to have happened in one instance in Ricketts and Wilder's experience (Wilder, 1911) and in one instance in the experience

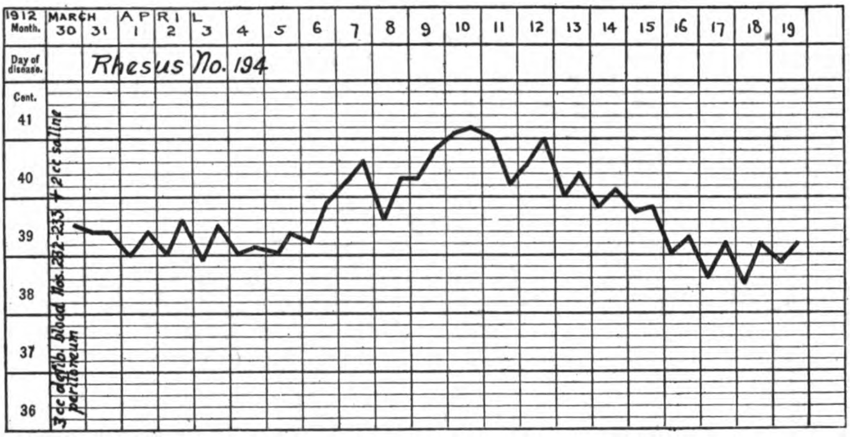
CHART No. 12



Temperature curve of rhesus No. 195, showing what was probably a reaction (complicated by tuberculosis) following an inoculation with blood serum.

of Nicolle, Conor, and Conseil (January, 1911). On subjecting this monkey to another immunity test (3 c. c. defibrinated blood intraperitoneally), however, 70 days after the inoculation with the filtered serum rhesus No. 194 responded promptly and sharply, as may be seen from chart 13.

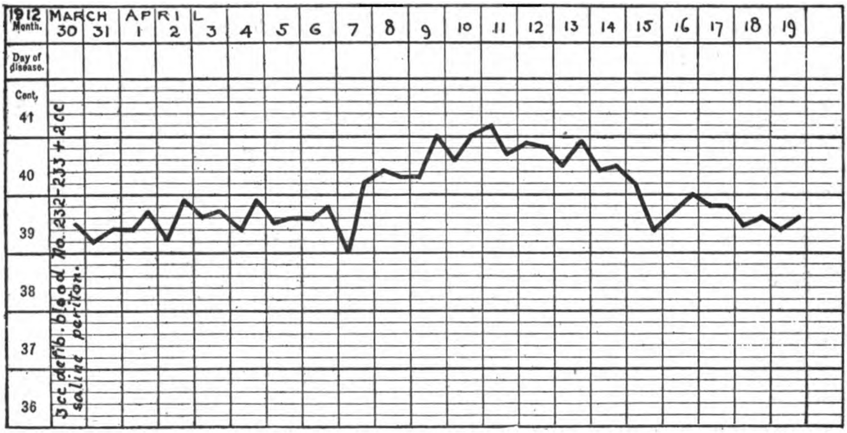
CHART No. 13



Temperature curve of rhesus No. 194, showing reaction following second immunity test.

It would appear therefore that the inoculation of rhesus No. 194 with 6 c. c. of filtered serum obtained after centrifugation of clotted typhus blood had neither infected nor vaccinated this animal.

CHART No. 14



Temperature curve of rhesus No. 223, showing reaction following immunity test.

*Experiment No. 11.*

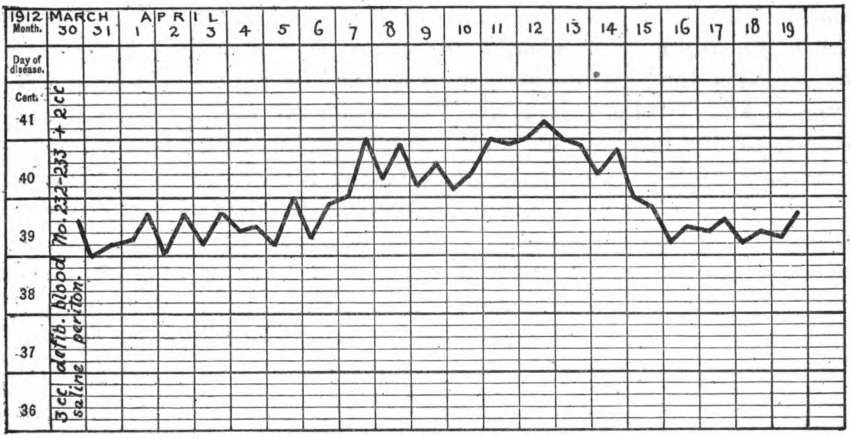
On March 6, 1912, blood was aspirated from rhesus No. 210 and No. 213. The former was in the seventh and the latter in the fifth day of an experimental typhus. The blood was allowed to clot. After three hours it was centrifugated in order better to separate the serum from the clot. The serum was then pipetted off and the two specimens were mixed. A portion of this serum was diluted with 3 volumes of saline solution and then passed through a Berkefeld filter, after which the following inoculations were made:

Rhesus No. 221 received 6 c. c. of unfiltered serum, rhesus Nos. 223 and 224 each received 6 c. c. of filtered serum intraperitoneally.

*Result:* None of these animals presented any evidence of a reaction following this inoculation. Twenty-four days later the three were subjected to an immunity test consisting of an intraperitoneal injection of 3 c. c. of virulent defibrinated blood of rhesus Nos. 232 and 233, diluted with 2 c. c. of saline solution. Following this rhesus Nos. 223 and 224 responded promptly and sharply (charts 14 and 15), but No. 221 gave no evidence of a reaction. Rhesus No. 221 has been subjected to two more immunity tests since, but so far has presented no evidence of a reaction. (Cf. Exp. 5.)

It would appear, therefore, that the inoculation of two monkeys with filtered serum obtained after centrifugation of clotted typhus blood had produced neither infection nor immunization in either.

CHART No. 15



Temperature curve of rhesus No. 224, showing reaction following immunity test.

Summarizing our attempts to infect the rhesus monkey with filtered typhus blood serum, we find that in no instance was infection produced, and that when subjected to an immunity test 2 (rhesus 115*a*, experiment 2, and rhesus 194, experiment 3) of the 7 monkeys employed at first appeared to be resistant (apparently vaccinated), but later when the test was repeated both responded.

Discussion.—Having presented first a summary of the results of the filtration experiments recorded in the literature and then detailed our own attempts to pass the virus of typhus through the Berkefeld filter, we may proceed with a consideration of their interpretation and significance.

The significance to be attached to the results of the filtration experiments, as a whole, evidently hinges on the interpretation to be given to the observation that some of the monkeys that had been inoculated with filtered typhus blood serum were subsequently resistant to an immunity test, although the inoculation with the filtrate had not been followed by any definite evidence of a febrile reaction. Nicolle, Conor, and Conseil (October, 1910, p. 3) interpret the resistance to the immunity test of one of their monkeys as indicating an immunization caused by " the microbe of typhus, which is very small and passes the Berkefeld filter"; whereas Wilder suggests that a similar result in Ricketts's and Wilder's experience " may be explained by one or more of several different hypotheses. The animal may have been naturally immune to typhus. If such is the case, however, he is the first normal monkey with which we have had to deal that has shown such an absolute immunity when inoculated with over 1 c. c. of virulent blood. A second possibility is that the animal was immunized by the filtered serum." " Such immunization could have been accomplished either by microorganisms sufficiently small to pass the filter, by fragments of organisms, or by toxins." Although Wilder is evidently disinclined to attach any importance to the idea that the normal monkey may be naturally immune to typhus, and Nicolle, Conor, and Conseil do not even consider it, the results of our inoculations with virulent typhus blood serum (unfiltered), as well as the final results of the repeated immunity tests, clearly show that something in the nature of a natural immunity or transient unresponsiveness to typhus is possessed by some normal monkeys and we believe this to be the true explanation of the instance noted by Nicolle, Conor, and Conseil and that by Ricketts and Wilder. We believe that, had they repeated the immunity tests in these monkeys as we did in ours, their results would probably have shown as did ours that these animals were only apparently (? transiently) resistant. These monkeys were therefore not immunized by the filtered typhus serum.

Conclusions.—It is permissible to conclude from the foregoing that there is no evidence to show that the virus in the blood of typhus is able to pass the Berkefeld filter; and, incidentally, that virulent typhus blood contains no toxin, or contains it in quantities too small to cause an appreciable increase in the normal resistance of the monkey when injected, even repeatedly, in the ordinary doses.

## THE VIRUS IN THE LOUSE.

The fact that the organism of typhus, as it occurs in the blood, does not appear capable of passing the Berkefeld filter does not of necessity mean that it may not be capable of passing in the form in which it exists in the body of the louse. It was decided, therefore, to put this idea to the test of experiment.

### *Experiment No. 12.*

On December 3, 1911, 82 living body lice of group No. 7—M and 83 of group No. 8—M were crushed and rubbed up in 16.5 c. c. of saline solution. Of this suspension 11 c. c. were diluted with an equal volume of saline solution and filtered through a Berkefeld filter. With 15 c. c. of the clear filtrate, representing 75 body lice, rhesus No. 311 was inoculated by an injection of about 13 c. c. intraperitoneally and about 2 c. c. subcutaneously. Control inoculations with the original (undiluted) suspension (reported in a previous communication by Goldberger and Anderson, Mar. 1, 1912) were made in two rhesus monkeys; one, No. 308, received 3.5 c. c. (representing 35 lice), and No. 309, 1.5 c. c. (representing 15 lice) subcutaneously.

Body lice of group No. 7—M had been allowed to feed daily during the 6 days immediately prior to the date of the experiment on various cases of typhus fever in the Hospital General, Mexico City. They were last applied to case No. 16—M, in the eleventh day of illness, at 10.50 to 11.20 a. m. December 2. Body lice of group No. 8—M were insects that had been allowed to feed daily during the 5 days immediately preceding the date of the experiment on various cases of typhus fever. They were last applied to case No. 16—M, in the eleventh day of illness, at 10.20 to 10.50 a. m. December 2. Throughout the period during which they were allowed to feed on patients with typhus the lice of groups Nos. 7—M and 8—M were kept at room temperature (about 14 to 24° C.).

*Result:* As has already been reported, the controls—rhesus Nos. 308 and 309—presented no evidence of a typhus reaction. No. 311 developed a markedly remittent temperature following the inoculation with the filtrate and later was found to be infected with tuberculosis. This experiment has, therefore, no significance, so far as filtration is concerned.

### *Experiment No. 13.*

The preceding experiment was repeated on December 10. At 5.30 p. m. 478 lice of group No. 9—M were crushed and rubbed up in 48 c. c. of saline solution. Of this suspension about 7.5 c. c. (representing about 73 lice) were subsequently injected in rhesus No. 320. Another portion of the suspension, after dilution with 2 volumes of saline solution, was filtered through a Berkefeld filter, and then 39 c. c. of the filtrate, representing not less than 130 lice, were injected intraperitoneally in rhesus No. 319.

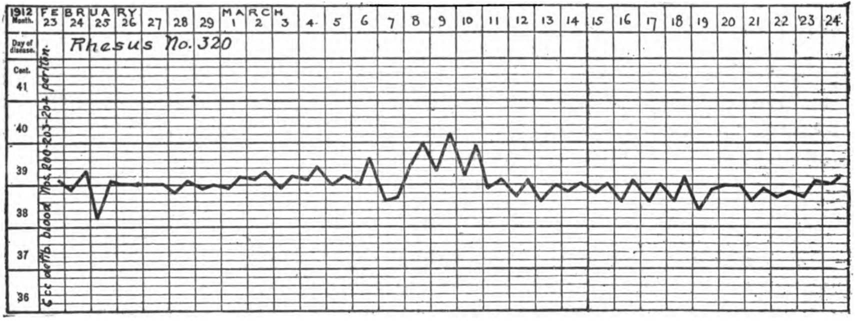
The lice composing group No. 9—M were body lice that had been applied daily and been allowed to feed on various cases of typhus at the Hospital General, Mexico City, during the six days immediately prior to the date of the experiment. Throughout this period they were kept at room temperature (14° to 24° C.). They were last applied on December 9 between 10.15 a. m. and 12 m. to case No. 19—M, in the seventh day of illness.

*Result:* As already reported (Goldberger and Anderson, 1912), rhesus No. 320 presented no evidence of a typhus reaction following the inoculation with the crushed lice suspension. This animal, after resisting two successive immunity tests with defibrinated blood, was subjected to a third test February 23, 1912, or 75 days after inoculation with the lice suspension. This consisted of an intraperitoneal inoculation of 6 c. c. of virulent defibrinated blood. During 3 days, beginning 14 days after this inoculation, rhesus No. 320 presented a well-defined perturbation in temperature that may have represented an abortive typhus reaction (chart 16).

Rhesus No. 319 presented no evidence of a reaction following the inoculation with the filtrate. Thirty-one days later he was subjected to an immunity test consisting of an intravenous inoculation of 3 c. c. of virulent defibrinated blood of rhesus No. 187; 21 days after this, having in the meantime presented no evidence of a typhus reaction, he was subjected to a second test consisting of an intravenous injection of 2.5 c. c. of virulent defibrinated blood of rhesus No. 115*a*. Not presenting any evidence of a reaction, he was subjected to a third test on February 23, 75 days after the inoculation with the filtered lice suspension. This test, as in the case of rhesus No. 320, consisted of an intraperitoneal injection of 6 c. c. of virulent defibrinated blood. During a period of observation of 33 days following this, rhesus No. 319 presented no recognizable indications of a typhus reaction.

DISCUSSION.—The immunity tests given monkeys No. 319 and No. 320 would indicate the possession of a complete insusceptibility to typhus by the former and an almost if not quite complete insusceptibility by the latter. In the early stages of our work we would have been inclined to interpret this resistance as due to an immunization induced by the original inoculation of filtered and unfiltered lice suspensions, respectively. Our more recent experience, however, having developed evidence that a healthy monkey may from time to time be encountered that is naturally resistant, a definite interpretation of the result of this single experiment is not permissible. Nevertheless, we undoubtedly have here a suggestion of a filterable phase in the body of the louse.

CHART NO. 16.



Temperature curve of rhesus No. 320, showing abortive (?) reaction following third immunity test.

With the idea of obtaining a clearer conception of the nature of the virus of typhus we have studied its resistance to drying and to certain extremes of temperature.

## DRYING.

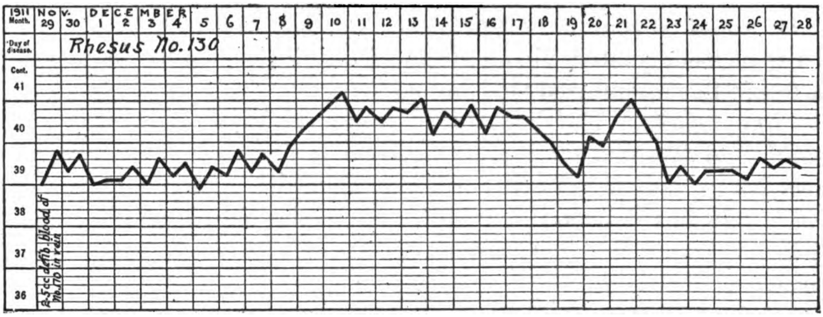
### *Experiment No. 14.*

At 12 m. October 22, 1911, 3 c. c. of virulent defibrinated blood of rhesus No. 141 (then in the third day of an experimental typhus) were put in one petri dish and 6 c. c. in another. The dishes with the lids tilted were placed over sulphuric acid in a desiccator from which the air was then exhausted. This apparatus with the blood was then placed at a temperature of from about 15° to 19° C. At the end of 25 hours the vacuum was broken and the dish containing the 3 c. c. of blood was removed, after which the air in the apparatus in which the dish with the 6 c. c. of blood remained was again exhausted and the apparatus continued as before.

The 3 c. c. of blood were found to have been dried and to have formed a scale on the bottom of the dish. The dry blood was rubbed up in saline solution and injected intraperitoneally into rhesus No. 130.

At the end of six days, that is, on October 28, the dish containing the 6 c. c. of blood was removed from the desiccator. The blood was found dried into a scale. This dried blood was rubbed up in saline solution and injected intraperitoneally into rhesus No. 129.

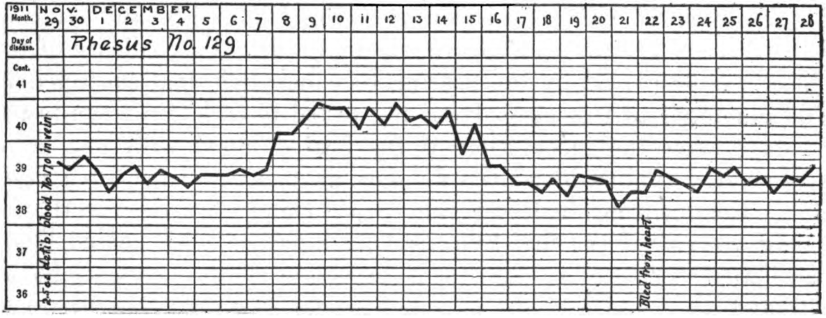
CHART NO. 17



Temperature curve of rhesus No. 130, showing reaction following immunity test.

*Result:* Neither rhesus No. 130 nor No. 129 presented any evidence of a reaction. Thirty-eight days after the inoculation of the former and 32 of the latter, both were subjected to an immunity test consisting of an intravenous injection of 2.5 c. c. of defibrinated blood of rhesus No. 170, then in the fourth day of an attack of typhus. Both monkeys promptly responded to this test, No. 130 developing a grave reaction and No. 129 a reaction of moderate severity (charts 17 and 18).

CHART NO. 18



Temperature curve of rhesus No. 129, showing reaction following immunity test.

Monkeys Nos. 130 and 129 were therefore neither infected nor immunized by the inoculation, in the one of 3 c. c. of virulent defibrinated blood after drying 25 hours, and in the other of 6 c. c. after drying 6 days.

### *Experiment No. 15.*

At 2 p. m., February 12, 1912, 3 c. c. of mixed virulent blood of rhesus Nos. 196, 198, and 322 were put in a petri dish, and the dish, with the cover tilted, placed over sulphuric acid in a desiccator as in experiment No. 14. After exhausting the air, the apparatus was placed at 15° C. At 2 p. m., February 13—that is, after 25 hours—the dish, with the blood now dried to a scale, was removed from the apparatus. The blood was then rubbed up in saline solution and injected intraperitoneally into rhesus No. 208.

*Result:* During a period of observation of 39 days monkey No. 208 presented no evidence of a reaction. Forty-six days after the inoculation this monkey was subjected to an immunity test consisting of an intraperitoneal injection of 3 c. c. of defibrinated blood of rhesus No. 234 diluted with 2 c. c. of saline solution. To this test monkey No. 208 reacted promptly and sharply.

It would appear, therefore, that the inoculation with 3 c. c. of virulent defibrinated blood after drying for 25 hours had neither infected nor immunized this animal.

CONCLUSIONS.—The negative outcome of these two experiments would indicate that the typhus virus can not survive drying, or, more accurately, that it loses its virulence when dried for 25 hours under the conditions described. Unfortunately the nature of the problem does not permit final conclusions to be drawn from so small a number of experiments.

## HEAT.

Gaviño and Girard were the first to study the resistance of the typhus virus to heat. In May, 1910, they inoculated a monkey with 10 c. c. of defibrinated typhus blood after heating at 50° for 40 minutes. The monkey so inoculated developed a well-marked febrile reaction after an incubation period of 14 days.

In a second experiment, performed three months later, they inoculated a monkey with 4 c. c. of defibrinated blood after heating at 55° for 15 minutes. This inoculation not being followed by any evidence of a reaction, Gaviño and Girard concluded that the virulence of the blood was destroyed by the heating to which they had subjected it. The immunity of this animal was not tested until later; when tested 56 days after the inoculation of the heated blood with 3 c. c. of defibrinated typhus blood the monkey gave no evidence of a reaction. Commenting on this result, Gaviño and Girard state that it would seem as if the first noninfecting injection with heated blood vaccinated against a subsequent injection with virulent blood, although they add that the objection might be raised that they had encountered in this animal one that was naturally immune.

In October, 1910, Nicolle, Conor, and Conseil reported an experiment in which the intraperitoneal injection of 4 c. c. of citrated blood (virulent for the controls) after heating at 50° C. for 15 minutes conferred no resistance to a subsequent immunity test. They concluded from this that the virus is destroyed by heating at 50° for 15 minutes. In view of the positive result obtained by Gaviño and Girard, and in the light of the fact several times referred to in other portions of this paper that the monkey is not invariably responsive to an infective inoculation, this conclusion is not permissible.

In a paper published in November, 1911, Gaviño and Girard report additional tests of the resistance of the typhus virus to heat. In one experiment they inoculated three guinea pigs intraperitoneally, each with 3 c. c. of defibrinated typhus blood heated at 55° for 15 minutes. None of these pigs presented any indications of a reaction. The immunity test, if made, is not recorded. In a second experiment they inoculated each of two monkeys with 7 c. c. of defibrinated typhus blood after heating at 55° for 15 minutes. Not presenting any evidence of a reaction, they were subjected to an immunity test one month later. To this both monkeys gave marked reactions. Consequently they conclude that typhus blood heated for 15 minutes at 55° loses its virulence and that a noninfecting injection of heated blood does not vaccinate against a subsequent inoculation with virulent blood.

The heating experiments recorded in the literature may be summarized as follows:

At 50° for 40 minutes.—One experiment, 1 monkey; blood retained its virulence (Gaviño and Girard).

At 50° for 15 minutes.—One experiment, 1 monkey; not infected, not immunized (Nicolle, Conor, and Conseil).

At 55° for 15 minutes.—One experiment, 1 monkey; not infected, but resisted immunity test (Gaviño and Girard). One experiment, 3 guinea pigs; not infected; immunity test not recorded (Gaviño and Girard). One experiment, 2 monkeys; not infected, not immunized (Gaviño and Girard).

Before attempting to interpret these results, we desire to record some experiments of our own.

### *Experiment No. 16.*

On October 22, 1911, we heated some virulent defibrinated blood of rhesus No. 141 in a tube at 55° for 15 minutes and with it inoculated 2 monkeys: Rhesus No. 131 received 3.5 c. c., and rhesus No. 132, 3 c. c., both intravenously.

*Result:* Neither of this pair of monkeys presented any evidence of a reaction following their inoculation, although 3 other monkeys, inoculated at the same time with like doses of the unheated blood, all developed well-marked typhus reactions. Twenty-nine days later rhesus Nos. 131 and 132 were subjected to an immunity test consisting in each of an intravenous injection of 2.5 c. c. of defibrinated blood of rhesus No. 166, then in the third day of a typhus reaction. Both animals reacted sharply (charts 19 and 20); rhesus No. 131, after an incubation of 14 days with a fever of 8 days' duration, and rhesus No. 132 after an incubation of 8 days with a fever of 6 days' duration.

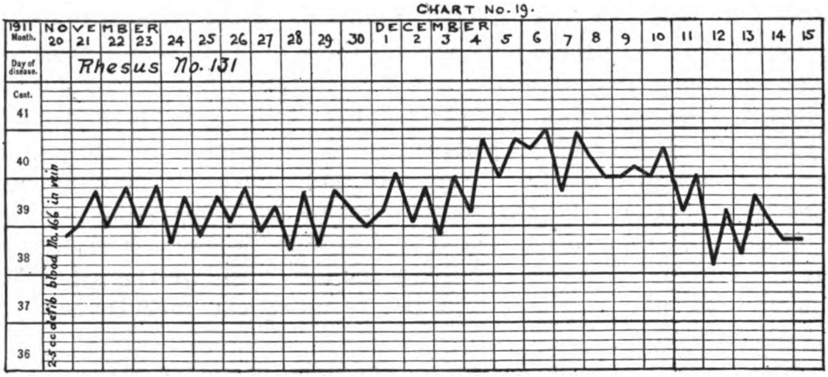
The two animals were therefore neither infected nor immunized.

### *Experiment No. 17.*

On February 14, 1912, we heated some virulent defibrinated blood in a tube at 60° for 5 minutes and injected 3 c. c. of it into the peritoneal cavity of rhesus No. 199.

*Result:* During a period of observation of 23 days monkey No. 199 gave no indications of a reaction. Tested at the end of this period by an intraperitoneal injection of 6 c. c. of virulent defibrinated blood, rhesus No. 199 developed a well-marked febrile reaction.

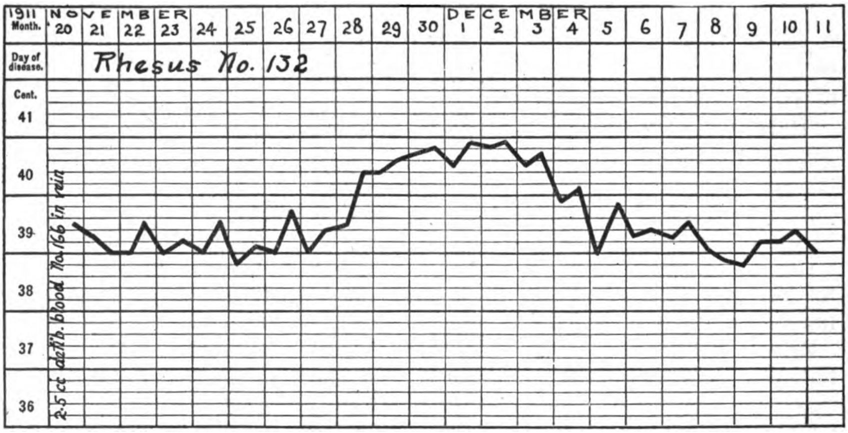
CHART No. 19.



Temperature curve of rhesus No. 131, showing reaction following immunity test.

This animal was therefore neither infected nor immunized by the injection of 3 c. c. of blood heated at 60° for 5 minutes.

CHART No. 20



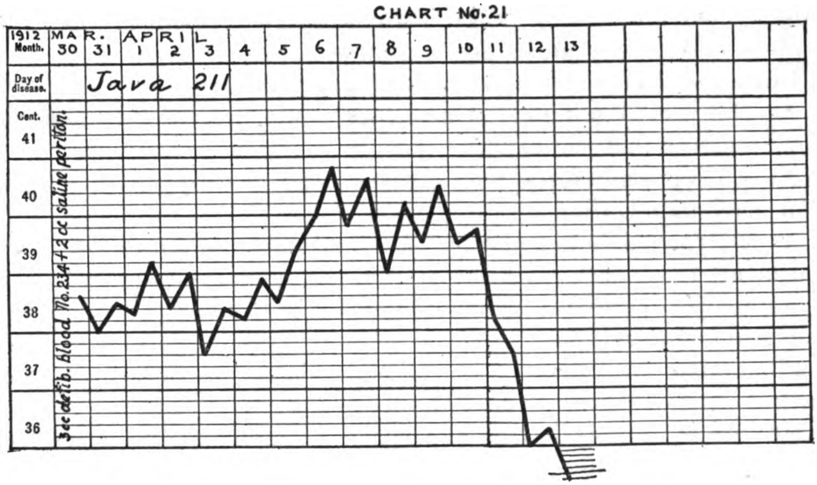
Temperature curve of rhesus No. 132, showing reaction following immunity test.

### *Experiment No. 18.*

On February 23 we gave Java monkey No. 211 two intraperitoneal injections of heated defibrinated typhus blood; the first of 6 c. c. heated by mistake at 60° for 5 minutes, and the second also of 6 c. c. heated at 55° for 5 minutes.

*Result:* Following these injections monkey No. 211 manifested no evidence of a reaction. An immunity test given 36 days later consisting of an intraperitoneal injection of 3 c. c. of defibrinated blood of rhesus No. 234, then in the second day of an experimental typhus, diluted with 2 c. c. of saline solution, was followed after an incubation period of 6 days by a typhus reaction terminating in death on the ninth day (chart 21).

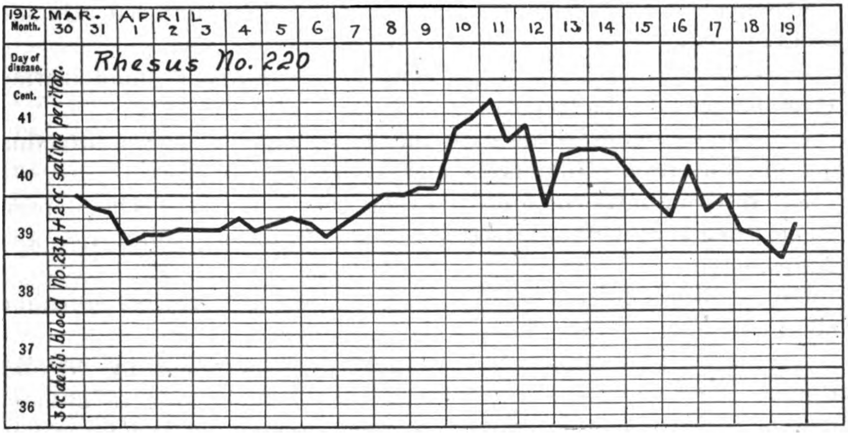
CHART No. 21



Temperature curve of Java No. 211, following immunity test.

It would appear therefore that this monkey was neither infected nor immunized by the intraperitoneal injection of 6 c. c. of blood heated at 55° for 5 minutes nor by that at 60° for 5 minutes.

CHART No. 22



Temperature curve of rhesus No. 220, showing reaction following immunity test.

*Experiment No. 19.*

On March 6, 1912, we heated some virulent blood in a tube at 55° for 5 minutes and injected 6 c. c. of it into the peritoneal cavity of rhesus No. 220.

*Result:* During a period of observation of 24 days this animal presented no recognizable indications of a reaction. Tested at the end of this time by an intraperitoneal injection of 3 c. c. of defibrinated blood of rhesus No. 234, diluted with 2 c. c. of saline solution, monkey No. 220 promptly developed a severe reaction (chart 22).

It would appear, therefore, that monkey No. 220 was neither infected nor immunized by the intraperitoneal injection of 6 c. c. of typhus blood heated at 55° for 5 minutes.

Our experiments may be summarized as follows:

At 55° for 15 minutes.—One experiment; neither infection nor immunization in 2 monkeys.

At 60° for 5 minutes.—One experiment, 1 monkey; neither infection nor immunization.

At 60° for 5 minutes and 55° for 5 minutes, combined.—One experiment, 1 monkey; neither infection nor immunization.

At 55° for 5 minutes.—One experiment, 1 monkey; neither infection nor immunization.

Combining the summary of the experiments previously recorded with that of our own, we have the following:

50° for 40 minutes.—One experiment, 1 monkey; blood retained its virulence (Gaviño and Girard).

50° for 15 minutes.—One experiment, 1 monkey; not infected, not immunized (Nicolle, Conor, and Conseil).

55° for 15 minutes.—One experiment, 1 monkey; not infected, but resisted immunity test (Gaviño and Girard).

One experiment, 3 guinea pigs; not infected; immunity test not reported (Gaviño and Girard).

One experiment, 2 monkeys; not infected, not immunized (Gaviño and Girard).

One experiment, 2 monkeys; neither infection nor immunization (Goldberger and Anderson).

55° for 5 minutes.—One experiment, 1 monkey; neither infection nor immunization (Goldberger and Anderson).

55° for 5 minutes combined with 60° for 5 minutes.—One experiment, 1 monkey; neither infection nor immunization (Goldberger and Anderson).

60° for 5 minutes.—One experiment, 1 monkey; neither infection nor immunization (Goldberger and Anderson).

Reviewing this summary, we find one experiment by Nicolle, Conor, and Conseil, in which blood heated at 50° for 15 minutes neither infected nor immunized, and one by Gaviño and Girard in which blood heated at 55° for 15 minutes did not infect but apparently immunized, which appear to be out of harmony with the other results. If we bear in mind the possibility, to which we have elsewhere in this paper called attention, that the normal monkey may at times fail to respond to the first and sometimes to the second inoculation with virulent material, we will have (what we believe is) the explanation of this lack of harmony.

Conclusions.—The determination of the resistance of typhus virus to heat is obviously beset with the same difficulties as the determination of its resistance to drying. Our inability to cultivate the organism hardly permits of the final determination of these points. Nevertheless, when we combine the results of our own heating experiments with those previously recorded we have a body of facts that justify certain conclusions that may be summarized as follows:

1. The virus may (perhaps generally does) retain its virulence after heating at 50° for 40 minutes.
2. The virus is deprived of virulence (? killed) by heating at 55° for 15 minutes.
3. The virus is probably deprived of virulence (? killed) by heating at 60° for 5 minutes.
4. The virus is probably deprived of virulence (? killed) by heating at 55° for 5 minutes.

## FREEZING.

So far as we are aware, the following attempts to determine the resistance of the typhus virus to "freezing" are the first to be recorded:

### *Experiment No. 20.*

At 12.30 p. m., October 22, 1911, some defibrinated blood of rhesus No. 141 (chart 3), then in the third day of a typhus reaction, was put into a freezing mixture of ice and hydrochloric acid and frozen solid. At 2.30 p. m. it was removed from this mixture and placed in a tank of brine at 0° C. At 1.30 p. m., October 23, it was taken out of the brine, rapidly thawed and, after warming slightly, 3 c. c. of it were intraperitoneally injected into rhesus No. 155.

*Result:* After an incubation period of 8 days this monkey developed a well-marked typhus reaction.

The infectivity of the blood was, therefore, not destroyed by "freezing" for 25 hours.

### *Experiment No. 21.*

On February 12, 1912, some virulent defibrinated blood from rhesus Nos. 196, 198, and 322 was frozen as in the preceding experiment and put in a tank of brine at 0° C. The blood was allowed to remain at this temperature for 8 days—that is, till February 20; it was then removed and allowed to thaw at room temperature. Having thawed, it was warmed slightly, and 6 c. c. of it was injected intraperitoneally into rhesus No. 210.

*Result* After an incubation period of 9 days, rhesus No. 210 developed a well-defined typhus reaction of about 8 days' duration. He later resisted an immunity test consisting of an intraperitoneal injection of 3 c. c. of defibrinated blood of rhesus No. 324, diluted with 2 c. c. of saline solution.

It appears, therefore, that the infectivity of the blood was not destroyed by "freezing" for 8 days.

### *Experiment No. 22.*

On February 23, 1912, some virulent blood obtained from rhesus Nos. 200, 203, and 204 was frozen and placed in brine at 0° C., as in the preceding experiments. It was kept in the brine at 0° C. for 15 days until March 9, when it was removed and permitted to thaw at room temperature. After warming slightly, 6 c. c. of it was injected intraperitoneally into Java monkey No. 226.

*Result:* During a period of observation of 30 days this animal presented no evidence of a reaction. Thirty-two days after the inoculation monkey No. 226 was subjected to an immunity test consisting of an intraperitoneal injection of 3 c. c. of defibrinated blood of rhesus No. 216, diluted with 2 c. c. of saline solution. To this it responded promptly with a severe typhus reaction.

Virulent blood, after "freezing" for 15 days, did not therefore infect or vaccinate.

CONCLUSIONS.—The foregoing experiments are not extensive enough to define more than roughly the degree of resistance of the typhus virus to freezing. It is clear, however, that typhus blood may retain its infectivity after freezing (0° C.) for at least 8 days.

## SUMMARY AND CONCLUSIONS.

1. The literature bearing on the duration of the infectivity of the blood is critically reviewed. Two experiments are reported, and the following conclusions are drawn:

(*a*) The blood of the monkey infected with typhus may be virulent in the prefebrile stage, but no satisfactory evidence of that fact has as yet been adduced.

(*b*) The blood of the monkey may still be virulent 24 to 32 hours after the return of the temperature to normal.

2. The literature bearing on the question of the localization of the virus is critically reviewed, and it is pointed out that the evidence adduced by Nicolle, Conor, and Conseil, in support of their hypothesis of an intraleucocytic localization, is not valid. New experiments are detailed, and the following conclusions are drawn:

(*a*) The available evidence favors the view that the typhus virus is extracellular and free in the circulating plasma.

(*b*) The serum of virulent typhus blood is constantly infective whether obtained from defibrinated blood or after clotting, instances of its apparent virulence being explicable by a natural resistance of the monkey.

(*c*) It may perhaps be possible to deprive typhus blood serum of its virulence by prolonged centrifugation; but this does not necessarily indicate an intraleucocytic localization of the virus.

(*d*) Repeated (three) washings of the blood corpuscles does not deprive them of their infectivity.

3. The literature bearing on the question of the filterability of the typhus virus is critically reviewed. It is found that eight attempts have been recorded to pass the virus through the Berkefeld filter. Of

these, six were clearly negative; in one of the other two, the monkey, without giving any evidence of a reaction to the inoculation, was later found to be resistant to an immunity test; in the other, one of a pair of monkeys is described as having presented a doubtful reaction to the inoculation and later was found resistant to a single immunity test.

New filtration experiments are reported, but in no instance was infection produced; when submitted to an immunity test, two of seven monkeys at first appeared to be resistant, but later, when the test was repeated, both responded.

The conclusion is drawn that there is no evidence to show that the virus in the blood of typhus is able to pass the Berkefeld filter; and, incidentally, that virulent typhus blood contains no toxin, or that it contains it in quantities too small to cause an appreciable increase in the normal resistance of the monkey when injected, even repeatedly, in the doses ordinarily employed for inoculating the monkey.

Two attempts are recorded to filter the virus as it exists in the body of the louse; in one, the monkey inoculated with the filtrate, without giving any indications of a reaction, was subsequently found refractory to repeated immunity tests, suggesting that he had been vaccinated by the filtrate. While this seems to point to the existence of a filterable phase in the body of the louse, it can not be regarded as conclusive without further corroborative work.

4. The resistance of the virus to drying is tested in two experiments with results indicating that the virus is deprived of virulence at the end of 25 hours.

5. The literature bearing on the resistance of the virus to heat is critically reviewed. The results recorded are summarized, some new experiments detailed, and the following conclusions drawn:

(*a*) The virus may (perhaps generally does) retain its virulence after heating at 50° for 40 minutes.

(*b*) The virus is deprived of virulence (? killed) by heating at 55° for 15 minutes.

(*c*) The virus is probably deprived of virulence (? killed) by heating at 60° for 5 minutes.

(*d*) The virus is probably deprived of virulence (? killed) by heating at 55° for 5 minutes.

6. The resistance of the virus to freezing (0° C.) is tested. It is found that it may retain its infectivity, after freezing (0° C.), for at least 8 days.

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# 7. STUDIES ON IMMUNITY AND MEANS OF TRANSMISSION OF TYPHUS.[1]

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## SUSCEPTIBILITY OF THE MONKEY.

Instances of transient or even permanent natural immunity of human beings to certain infections are not unknown. Most of these are clinical observations. It has been frequently noted that of a number of persons of a family or of a community presumably equally exposed some escape. Those who have made many vaccinations for the prevention of smallpox, especially of children, are familiar with the fact that sometimes an individual may resist several repeated inoculations; this, while it may frequently be attributed to a non-potent virus, is, in some instances at least, undoubtedly due to a more or less transient natural immunity to vaccinia.

An interesting instance of natural immunity to an infectious disease in the human subject is reported by Reed (1901). One of the subjects experimented on by Reed and Carroll resisted a subcutaneous injection of 1.5 c. c. of yellow fever blood and later, also, the bites of some infected mosquitoes.

Similar examples of individual resistance of animals to experimental infection have been noted. In the course of our studies on measles we found quite a marked variation in the susceptibility of different individuals. Marks has well brought out the variation in susceptibility of young rabbits to poliomyelitis; and Dorset, McBryde, and Niles report instances of natural immunity in the hog to hog cholera. Metchnikoff and Besredka (March, 1911) report that in 1 of 16 experiments they failed to infect the chimpanzee with typhoid; and McCoy and Chapin (January, 1912) report that a considerable percentage of San Francisco rats were found by them to be immune to plague at a time when immunity, due to a previous attack, could reasonably be excluded. Coming to typhus, we find that Nicolle was unsuccessful in his initial attempts to infect monkeys directly from the human subject and from his experience concluded that to obtain success the virus had to be prepared by passage through a higher ape, the chimpanzee. Our own work and that of Ricketts and Wilder, of Gaviño and Girard, and others and more recently that of Nicolle himself has shown that such preparatory treatment of the virus is unnecessary. The apparently ready susceptibility of the monkey encountered by us in our early work suggested to us that Nicolle's initial lack of success was due either to the small amounts of blood used, to the subcutaneous route employed by him in those inoculations, or to both combined. Apart from these factors there appeared to us then no reason to suspect natural resistance as playing a part in explaining Nicolle's negative results.

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[1] Manuscript submitted for publication July 30, 1912.

Gaviño and Girard appear to have been the first to suggest that some monkeys may be naturally immune to typhus. They reported that a monkey of the species *Ateles vellerosus* that had previously received an injection of heated typhus blood failed to present any reaction following an immunity test of 3 c. c. of virulent blood. They suggest that instead of the animal having been vaccinated by the first noninfecting injection it may have been naturally immune to typhus.

Dreyer (1911) reports that in the course of his work he found one monkey (*cercopithecus?*) entirely refractory. This animal showed no trace of illness, although two others treated with the same material at the same time and in substantially the same dose promptly developed the usual (typhus) symptoms. He believed himself justified in interpreting this as a case of natural immunity. He does not state whether he tested the susceptibility of this animal a second time.

Wilder reports that a monkey that had received an injection of filtered typhus blood serum failed to react when given an immunity test of 4 c. c. of typhus virulent blood and suggests, among other possibilities, that the animal may have been naturally immune.

Nicolle and his coworkers, in their interpretations, do not appear to have considered the possibility of a natural immunity in the monkey. We believe, however, that a critical study of their reported protocols shows that they had encountered the same phenomenon.

In the course of our recent work with both the New York and the Mexican virus we obtained results that brought the possibility of the occurrence of a natural resistance to typhus forcibly to our attention.

In Table No. 1 we summarize all our monkey inoculations in which the first or primary inoculation was with monkey-typhus blood. It is to be understood that in these and other inoculations the infectivity of the blood, unless otherwise stated, was always proven by appropriate controls.

### TABLE No. 1.—*Initial inoculation with monkey typhus blood.*[1]

| Monkey No. | Date. | C. c. inoculated. | Source. | Site. | Incubation period (days). | Duration of disease (days). | Result. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| Rhesus 95. | Oct. 22, 1911 | 3.5 | Rhesus 141–[3–?][2] | Vein | 6 | 14 | + | |
| | Nov. 23, 1911 | 3 | Rhesus 306–[3–8] | Vein | 0 | 0 | − | |
| | Dec. 8, 1911 | 3.5 | Case 19M[3]–[6–14] | Peritoneum | 0 | 0 | − | |
| Bonnet 125. | Oct. 22, 1911 | 2.5 | Rhesus 141–[3–?] | Vein | 10 | 10 | + | |
| Rhesus 128. | Oct. 25, 1911 | 3 | Rhesus 139–[3–10] | Vein and subcutaneous. | 6 | 9 | + | |
| | Nov. 13, 1911 | 3 | Rhesus 162 | Vein | 0 | 0 | − | Washed corpuscles; probably not infective. |
| Rhesus 139. | Oct. 13, 1911 | 3.5 | Rhesus 158–[1–9] | Vein and subcutaneous. | 10 | 10 | + | |
| | Nov. 13, 1911 | 3 | Rhesus 162 | Peritoneum | 0 | 0 | − | Do. |
| Rhesus 140. | Oct. 13, 1911 | 3.5 | Rhesus 158–[1–9] | Vein | 10 | 9 | + | |
| | Nov. 13, 1911 | 3 | Rhesus 162 | Peritoneum | 0 | 0 | − | Do. |
| Rhesus 146. | Nov. 5, 1911 | 2 | Rhesus 148–[6–10] | Vein | 9 | 10 | + | |
| Rhesus 158. | Oct. 5, 1911 | 3 | Rhesus 150–[6–13] | Peritoneum | 8 | 9 | + | |
| | Nov. 9, 1911 | 5 | Case 1M | Vein and subcutaneous. | 0 | 0 | − | |
| | Dec. 2, 1911 | 2.5 | Case 16M | Vein | 0 | 0 | − | |
| | Dec. 22, 1911 | 4.5 | Case 26M | Peritoneum | 0 | 0 | − | |
| Rhesus 159. | Oct. 22, 1911 | 3.5 | Rhesus 141–[3–?] | Vein and peritoneum. | 0 | 0 | − | Spotted fever immune. |
| | Nov. 20, 1911 | 2.5 | Rhesus 166–[3–12] | Vein | 10 | 9 | + | |
| Rhesus 163. | Oct. 31, 1911 | 3 | Rhesus 95–[4–14] | Vein | 10 | 8 | + | |
| Rhesus 164. | do | 3.5 | do | Vein and subcutaneous. | 10 | 10 | + | |
| Rhesus 166. | Nov. 2, 1911 | 2.5 | Rhesus 163–[2–8] | Vein | 7 | 12 | + | |
| Rhesus 167. | Nov. 11, 1911 | 3 | Rhesus 163 | Subcutaneous | 12 | 8 | + | |
| Rhesus 170. | Nov. 30, 1911 | 2.5 | Rhesus 166–[3–12] | Vein | 6 | 9 | + | |
| Rhesus 171. | Nov. 20, 1911 | 2.5 | do | Subcutaneous | 0 | 0 | − | |
| | Dec. 23, 1911 | 2.5 | Rhesus 176–[2–10] | Vein | 9 | 9 | + | |
| Rhesus 174. | Nov. 29, 1911 | 2.5 | Rhesus 170–[4–9] | Vein | 10 | 12 | + | |
| Rhesus 175. | do | 2.5 | do | Vein | 9 | 9 | + | |
| Rhesus 176. | Dec. 12, 1911 | 2.5 | Rhesus 161–[7–15] | Vein | 10 | 10 | + | |
| Rhesus 177. | do | 2.5 | Rhesus 175–[5–10] | Vein | 9 | 11 | + | |
| Rhesus 186. | Jan. 2, 1912 | 3 | Rhesus 171–[2–9] | Vein | 5 | 11 | + | |
| Rhesus 187. | do | 2.5 | do | Vein | 7 | 6 | + | |
| Rhesus 189. | Jan. 10, 1912 | 3.25 | Rhesus 187–[2–6] | Vein | 0 | 0 | − | |
| | Jan. 31, 1912 | 3.5 | Rhesus 115*a*–[4–7] | Vein and peritoneum. | 0 | 0 | − | |
| | Feb. 23, 1912 | 6 | Rhesus 200, 203, 204. | Peritoneum | 0 | 0 | − | |
| | Mar. 30, 1912 | 3 | Rhesus 232, 233 | Peritoneum | 0 | 0 | − | |
| | Apr. 20, 1912 | 3 | Rhesus 225–[5–13] | Peritoneum | 0 | 0 | − | |
| | May 4, 1912 | 3 | Rhesus 229 | Peritoneum | 0 | 0 | − | |
| | May 9, 1912 | 3.5 | G. P. 70, 71, 72, 73 | Peritoneum | 0 | 0 | − | |
| | May 31, 1912 | 14 | Rhesus 258 | Peritoneum | 0 | 0 | − | |
| Rhesus 190. | Jan. 10, 1912 | 3 | Rhesus 187–[2–7] | Vein | 8 | 13 | + | |
| Rhesus 192. | Jan. 20, 1912 | 3 | Rhesus 308, 309–[2–7]. | Vein | 12 | 8 | + | |
| Rhesus 196. | Jan. 31, 1912 | 2.5 | Rhesus 115*a*–[4–7] | Vein | 9 | 7 | + | |

[1] Defibrinated unless otherwise stated.

[2] The first number in brackets indicates the day of disease, and the second, when given, the duration of illness of the animal; so that under Rhesus No. 128, for instance, we should read "Given 3 c. c. blood from Rhesus No. 139 on the third day of a fever lasting 10 days."

[3] M=Mexican strain.

**TABLE No. 1.**—*Initial inoculation with monkey typhus blood*—Continued.

| Monkey No. | Date. | C. c. inoculated. | Source. | Site. | Incubation period (days). | Duration of disease (days). | Result. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| Rhesus 197. | Jan. 31, 1912 | 2.5 | Rhesus 115*a*-[4-7]. | Vein | 0 | 0 | – | |
| | Feb. 23, 1912 | 6 | Rhesus 200, 203, 204. | Subcutaneous | .... | .... | – | |
| | Mar. 30, 1912 | 3 | Rhesus 232, 233 | Peritoneum | 7 | 5 | + | |
| Rhesus 198. | Feb. 1, 1912 | 2.5 | Rhesus 184-[4-14] | Vein | 9 | 8 | + | |
| Rhesus 200. | Feb. 12, 1912 | 2.5 | Rhesus, 196, 198, 322. | Peritoneum | 7 | 11 | + | |
| Rhesus 201. | .....do | 4 | .....do | Peritoneum | 0 | 0 | – | |
| | Mar. 6, 1912 | 6 | Rhesus 213, 312 | Peritoneum | .... | .... | (1) | |
| | Mar. 30, 1912 | 3 | Rhesus 232, 233 | Peritoneum | 0 | 0 | – | |
| | Apr. 20, 1912 | 3 | Rhesus 225 | Peritoneum | 0 | 0 | – | |
| | May 4, 1912 | 3 | Rhesus 229 | Peritoneum | 0 | 0 | – | |
| Rhesus 203. | Feb. 12, 1912 | 6 | Rhesus 196, 198, 322. | Peritoneum | 7 | 8 | + | |
| Rhesus 204. | .....do | 6 | .....do | Peritoneum | 7 | 10 | + | |
| Rhesus 206 | .....do | ...... | .....do | Peritoneum | 7 | 9 | + | "Cream layers." |
| Rhesus 207. | .....do | 3 | .....do | Peritoneum | 9 | 8 | + | Washed corpuscles. |
| Rhesus 212. | Feb. 23, 1912 | 6 | Rhesus 200, 203, 204. | Peritoneum | 7 | 9 | + | |
| Rhesus 213. | Feb. 23, 1912 | 6 | .....do | Peritoneum | 8 | 8 | + | |
| Rhesus 218. | Mar. 6, 1912 | 4 | Rhesus 213, 312 | Peritoneum | 11 | 9 | + | |
| Rhesus 231 | Mar. 20, 1912 | 7 | Rhesus 218-[4-9] | Peritoneum | 0 | 0 | – | |
| | Apr. 20, 1912 | 3 | Rhesus 225-[5-13] | Peritoneum | 9 | 8 | + | |
| Rhesus 232. | Mar. 20, 1912 | 2.5 | Rhesus 199-[6-12] | Peritoneum | 9 | 9 | + | |
| Rhesus 233. | .....do | 10 | .....do | Peritoneum | 9 | 8 | + | |
| Rhesus 238. | Mar. 30, 1912 | 3 | Rhesus 232, 233 | Peritoneum | 8 | 9 | + | |
| Rhesus 244. | Apr. 10, 1912 | 3 | Rhesus 216-[5-12] | Peritoneum | 6 | 8 | + | |
| Rhesus 250. | Apr. 20, 1912 | 3 | Rhesus 225-[5-13] | Peritoneum | 8 | (3) | + | |
| Rhesus 252. | Apr. 23, 1912 | 3 | Rhesus 225-[8-13] | Peritoneum | 9 | 10 | + | |
| Rhesus 253. | .....do | 3 | .....do | Peritoneum | 9 | 16 | + | |
| Rhesus 256. | May 4, 1912 | 3 | Rhesus 229-[6-8] | Peritoneum | 0 | 0 | – | |
| | May 31, 1912 | 3 | Rhesus 258 | Peritoneum | 0 | 0 | – | |
| Rhesus 257. | May 4, 1912 | 3 | Rhesus 229-[6-8] | Peritoneum | 21 | 6 | + | |
| Rhesus 314. | Nov. 23, 1911 | 3 | Rhesus 306-[3-8] | Vein | 0 | 0 | – | Infectivity of blood 306 not checked by control. |
| | Dec. 22, 1911 | 4.5 | Case 26M-[12-18] | Peritoneum | 7 | 8 | + | |

[1] Abortive(?).

By reference to this table it will be found that 46 monkeys were given a primary inoculation of virulent blood, and to this 8, or 17.4 per cent, failed to react; these 8 were given a second inoculation, to which 4, or 8.7 per cent, of the original 46 failed to respond. It is right to state, however, that one of these 4, namely, rhesus No. 201 (chart No. 1), may perhaps be considered to have presented a suggestion of an "abortive" reaction that had its acme on March 26. So slight is this, however, that it escaped our attention at the time of its occurrence. If, for the sake of conservatism, we count this as a positive reaction, we have only 3 of the 8 that failed to respond to a second inoculation; or, in other words, at least 6.5 per cent of the monkeys failed to respond to two separate inoculations with virulent blood.

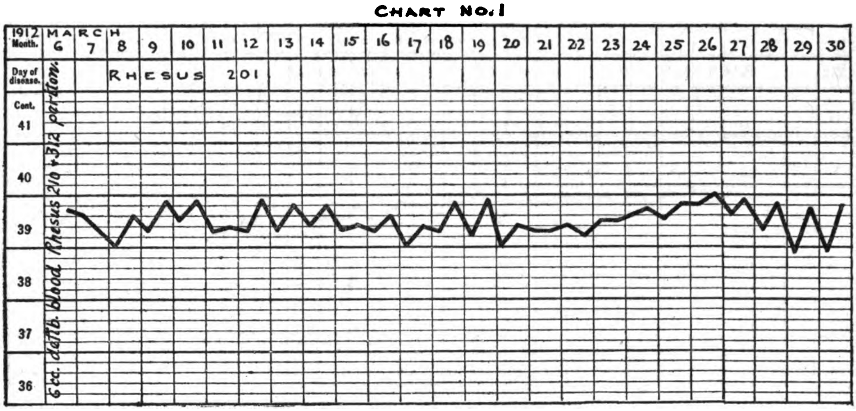

CHART No. 1.—Temperature curve of rhesus No. 201 following second inoculation, showing what may have been an "abortive" reaction with its acme on March 26.

Of the three clearly resistant to the second inoculation, the immunity of only two was further tested. Of these one, rhesus No. 197, although given less than half its previous dose, now developed a mild though well-defined febrile reaction (charts Nos. 2, 3, and 4); the other, rhesus No. 189, presented no indications of a reaction. After this, rhesus No. 189 was subjected to five more tests. In all, between January 2 and June 29, this animal received eight inoculations with virulent blood ranging in amount from 3 to 14 c. c., to which no recognizable evidence of a reaction was noted at any time during the period of observation. (See charts Nos. 5–11.)

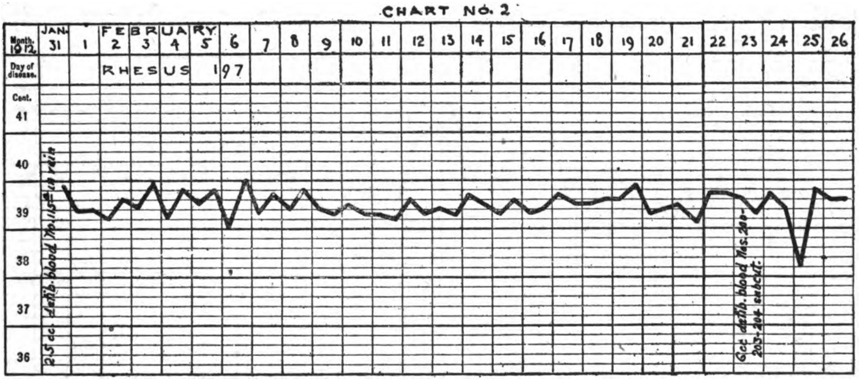

CHART No. 2.—Temperature curve of rhesus No. 197 after initial inoculation; result negative.

Of 46 animals, therefore, at least one (2.2 per cent) has failed to respond to any of the eight inoculations to which it was subjected.

In Table No. 2 we present a summary of our inoculations in which the initial one was with either unfiltered or filtered serum.

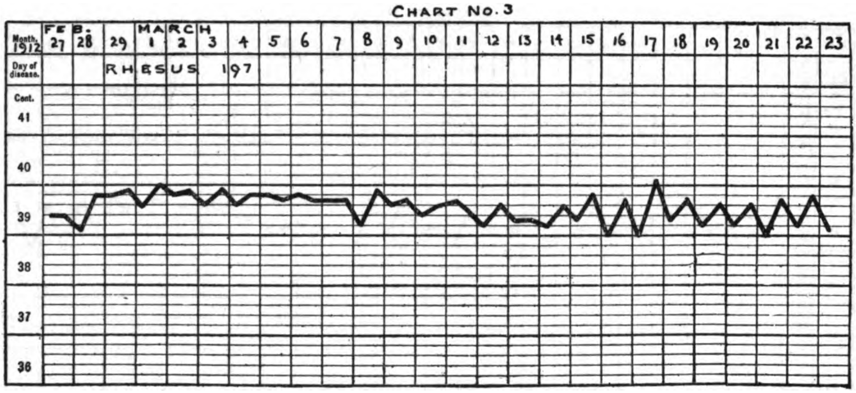

CHART No. 3.—Temperature curve of rhesus No. 197 after second inoculation; result negative.

Five monkeys received a first inoculation of virulent unfiltered serum; three of the five animals failed to respond to this inoculation, of these three, two responded promptly to the first immunity test (second virulent inoculation), while one, rhesus No. 221 (charts Nos. 12–16), has so far resisted five successive immunity tests (six virulent inoculations).

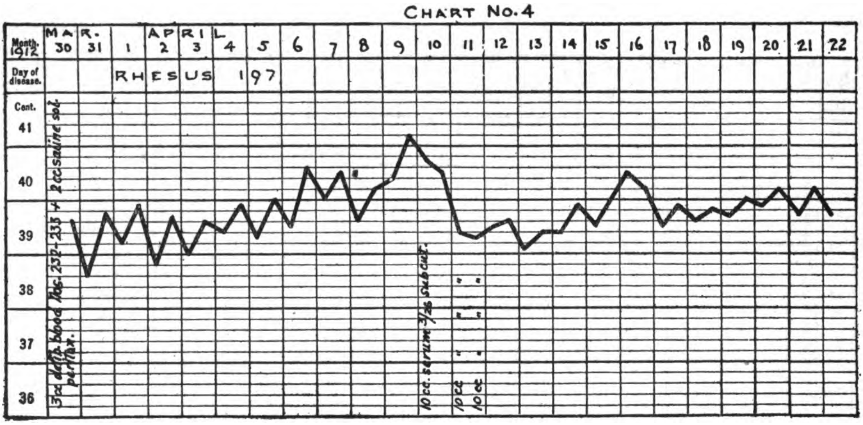

CHART No. 4.—Temperature curve of rhesus No. 197, to show typhus reaction after third inoculation, first and second having been negative.

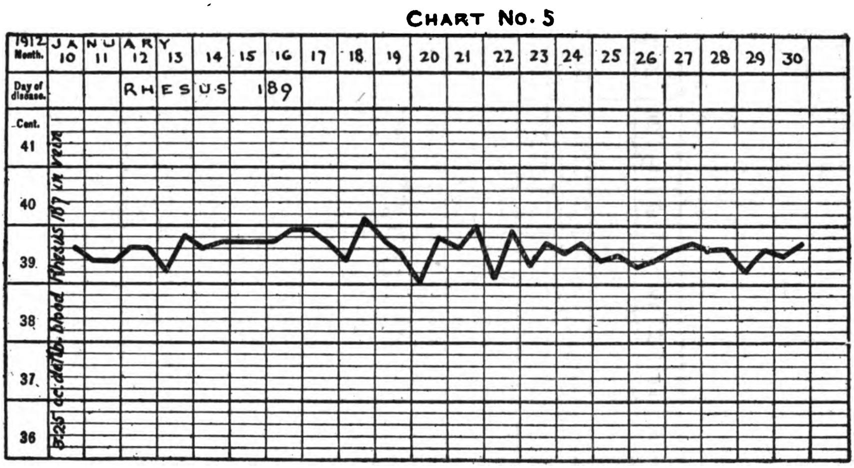

**Chart No. 5.**—Temperature curve of rhesus No. 189, to show resistance to repeated inoculations of virulent typhus blood; natural immunity.

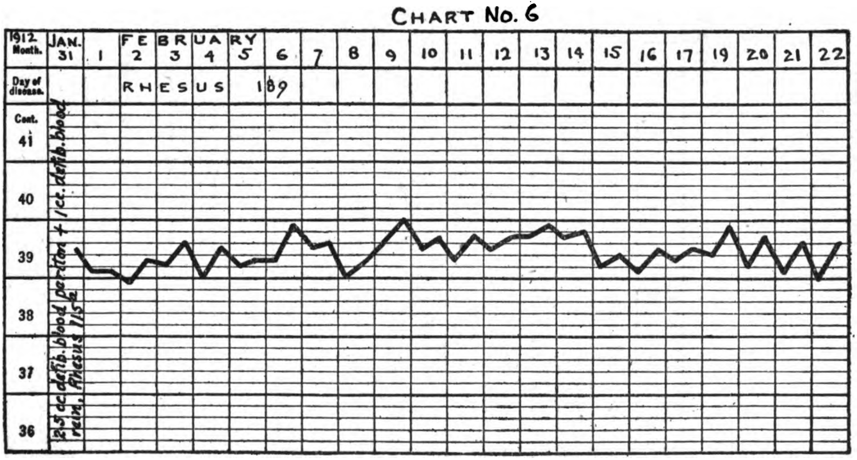

**Chart No. 6.**—Temperature curve of rhesus No. 189, to show resistance to repeated inoculations of virulent typhus blood; natural immunity.

## CHART No. 7

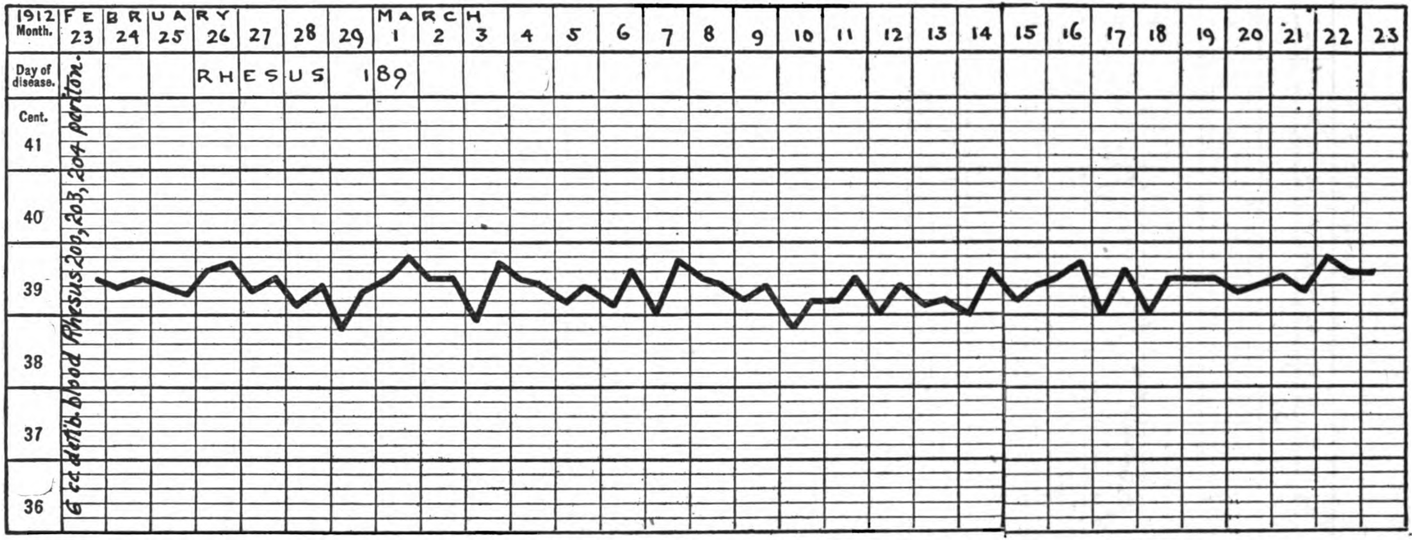

CHART No. 7.—Temperature curve of rhesus No. 189, to show resistance to repeated inoculations of virulent typhus blood; natural immunity.

CHART NO. 8.

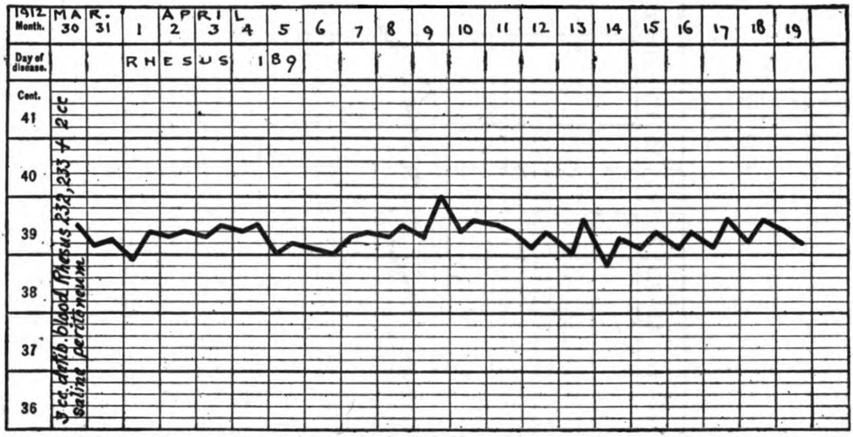

CHART No. 8.—Temperature curve of rhesus No. 189, to show resistance to repeated inoculations of virulent typhus blood; natural immunity.

CHART NO. 9

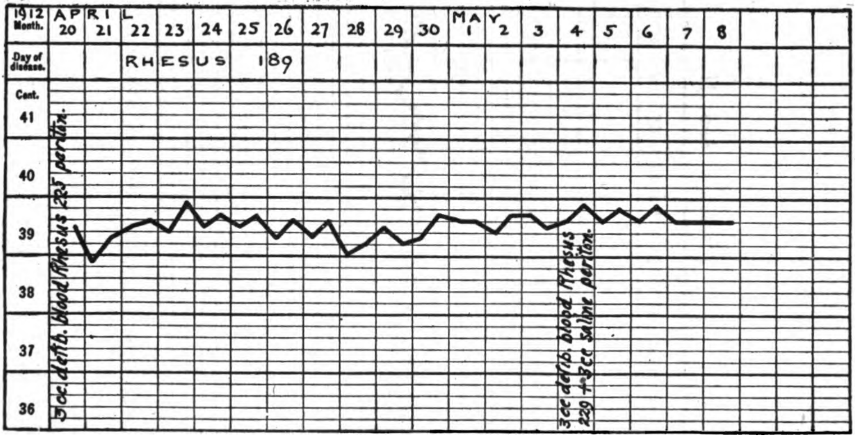

CHART No. 9.—Temperature curve of rhesus No. 189, to show resistance to repeated inoculations of virulent typhus blood; natural immunity.

CHART NO. 10

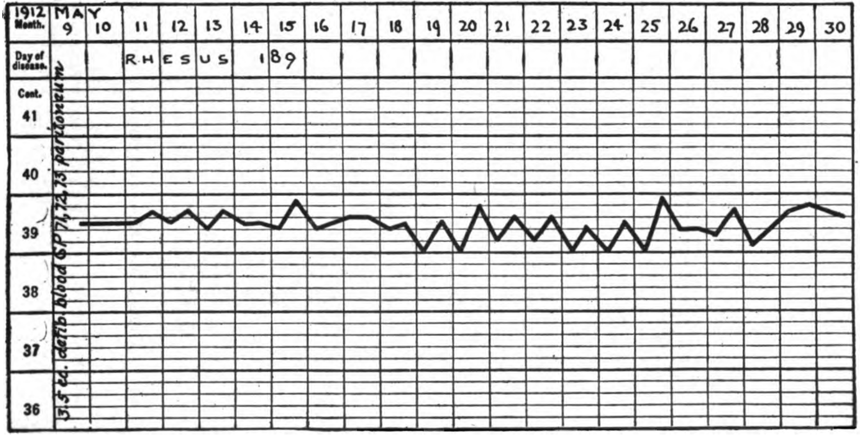

CHART No. 10.—Temperature curve of rhesus No. 189, to show resistance to repeated inoculations of virulent typhus blood; natural immunity.

## CHART No. 11

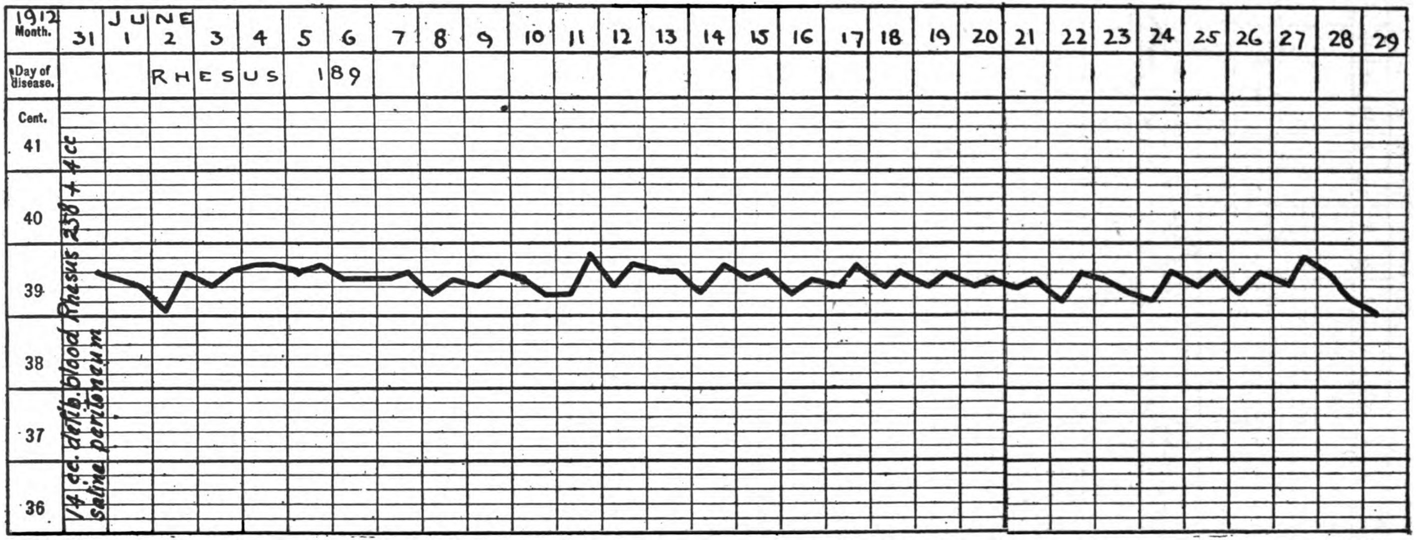

**Chart No. 11.**—Temperature curve of rhesus No. 189, to show resistance to repeated inoculations of virulent typhus blood; natural immunity.

CHART No. 12

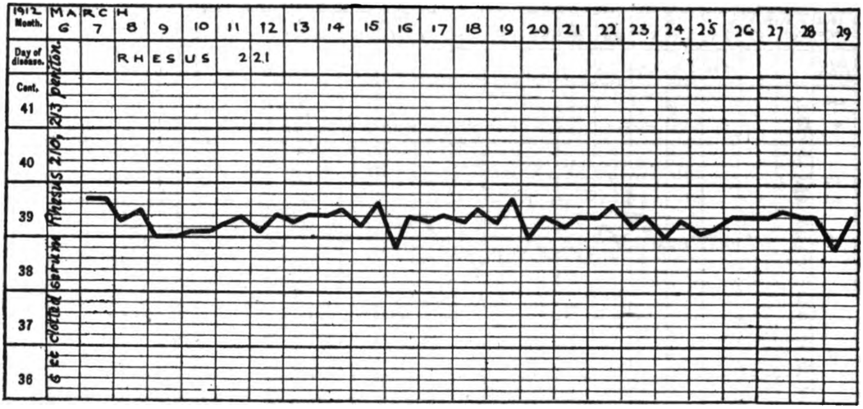

**Chart** No. 12.—Temperature curve of rhesus No. 221, to show resistance to repeated inoculation of virulent serum or blood; natural immunity.

CHART No. 13

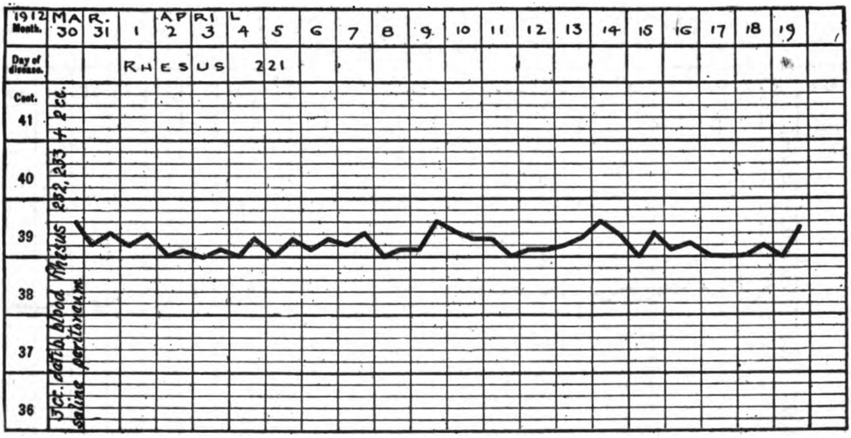

**Chart** No. 13.—Temperature curve of rhesus No. 221, to show resistance to repeated inoculation of virulent serum or blood; natural immunity.

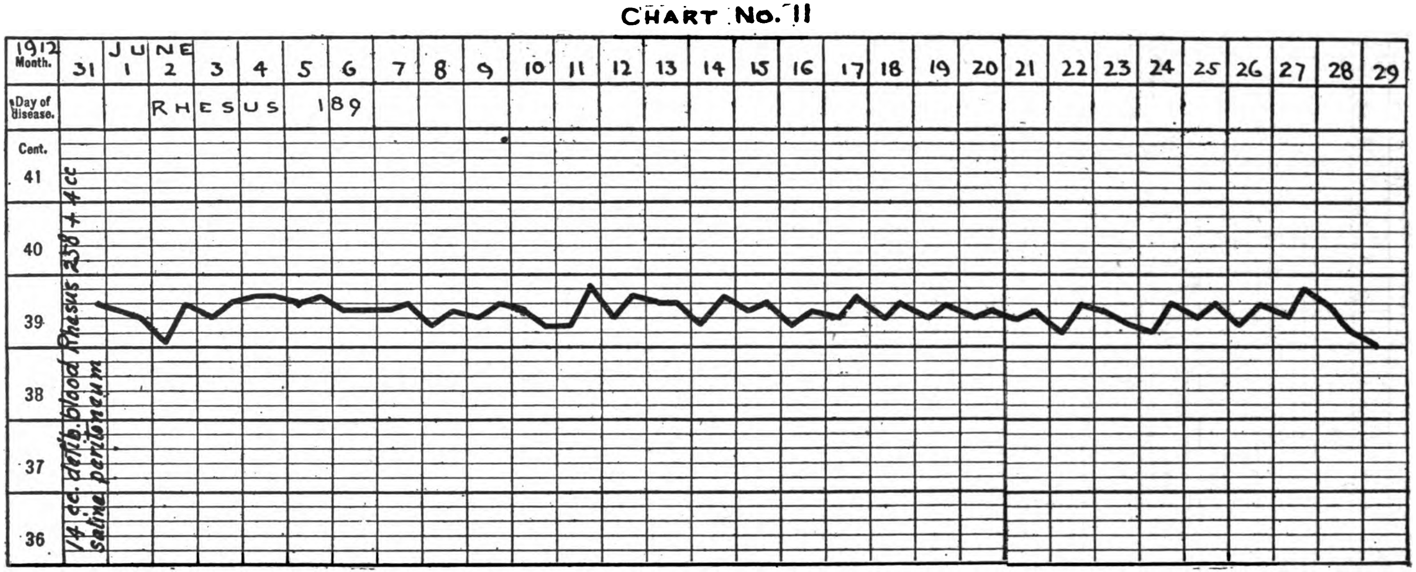

CHART No. 11.—Temperature curve of rhesus No. 189, to show resistance to repeated inoculations of virulent typhus blood; natural immunity.

CHART No. 12

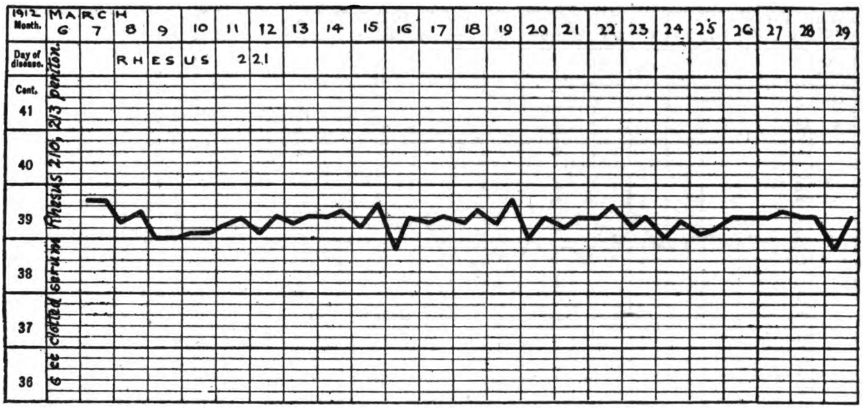

**CHART** No. 12.—Temperature curve of rhesus No. 221, to show resistance to repeated inoculation of virulent serum or blood; natural immunity.

CHART No. 13

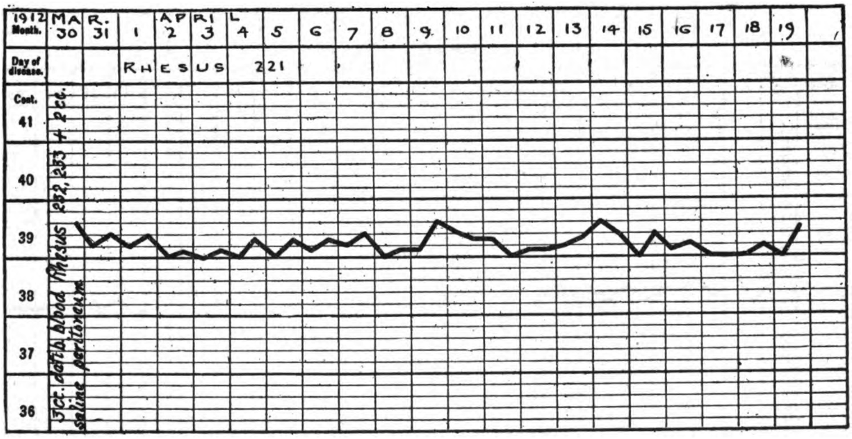

**CHART** No. 13.—Temperature curve of rhesus No. 221, to show resistance to repeated inoculation of virulent serum or blood; natural immunity.

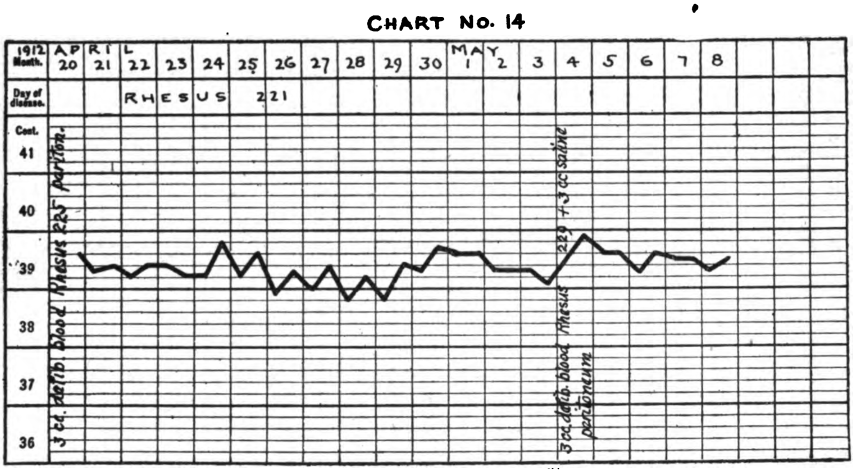

**Chart** No. 14.—Temperature curve of rhesus No. 221, to show resistance to repeated inoculation of virulent serum or blood; natural immunity.

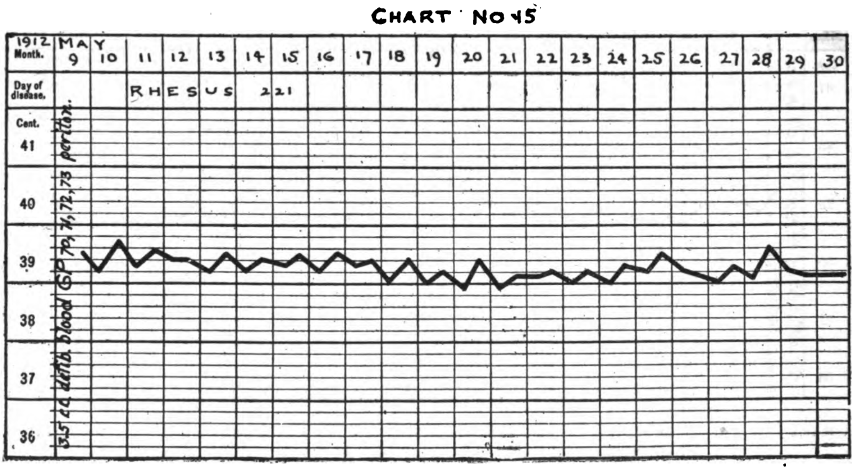

**Chart** No. 15.—Temperature curve of rhesus No. 221, to show resistance to repeated inoculation of virulent serum or blood; natural immunity.

## CHART No. 16

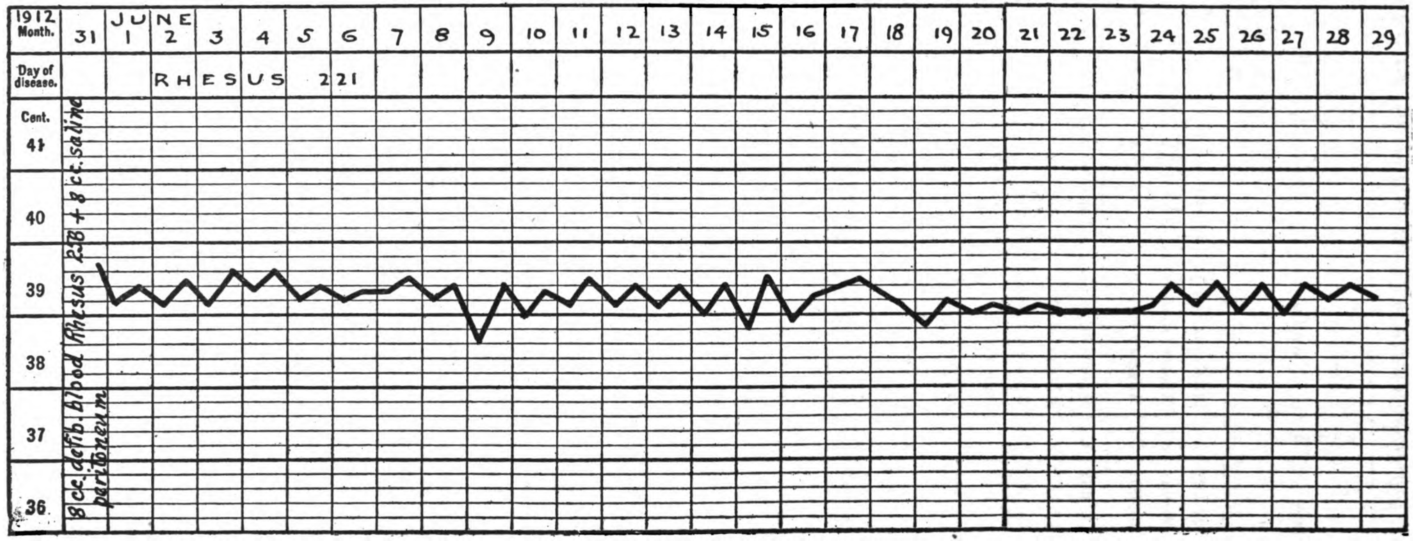

CHART No. 16.—Temperature curve of rhesus No. 221, to show resistance to repeated inoculation of virulent serum or blood; natural immunity.

**Table** No. 2.—*Initial inoculation with virulent serum, filtered or unfiltered.*

| Monkey No. | Date. | C. c. inoculated. | Source. | Site. | Incubation period (days). | Duration of disease (days). | Result. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| Rhesus 115A | Oct. 22, 1911 | 3 | Rhesus 141–[3–.][1] | Peritoneum and vein. | 0 | 0 | – | Filtered serum. |
|  | Nov. 20, 1911 | 2.5 | Rhesus 166–[3–12] | Subcutaneous. | 0 | 0 | – | Defibrinated blood. |
|  | Dec. 23, 1911 | 2.5 | Rhesus 176–[2–10] | Subcutaneous and vein. | 0 | 0 | – | Do. |
|  | Jan. 20, 1912 | 3.5 | Rhesus 308, 309–[2–7]. | Peritoneum. | 8 | 8 | + | Do. |
| Rhesus 115B | Oct. 22, 1911 | 3 | Rhesus 141–[3–.] | Peritoneum and vein. | 0 | 0 | – | Filtered serum. |
|  | Nov. 20, 1911 | 2.5 | Rhesus 166–[3–12] | Vein | 6 | 10 | + | Defibrinated blood. |
|  | Dec. 30, 1911 | 6 | Case 38M [2] | Peritoneum. | 0 | 0 | – | Do. |
|  | Jan. 1, 1912 | 4 | Case 39M |  |  |  |  |  |
| Rhesus 141 | Oct. 13, 1911 | 3.5 | Rhesus 158–[1–9] | Vein | 7 | .... | + | Accidentally killed third day. |
| Rhesus 142 | .....do | 4.5 | .....do | Peritoneum and subcutaneous. | 0 | 0 | – | Serum. |
|  | Nov. 11, 1911 | 2.5 | Rhesus 163–[2–8] | Vein | 6 | 7 | + | Defibrinated blood. |
|  | Nov. 29, 1911 | 5 | Rhesus 170–[4–9] | Vein | 0 | 0 | – | Do. |
|  | Dec. 12, 1911 | 4 | Rhesus 161–[7–15] | Vein | 0 | 0 | – | Do. |
|  | Jan. 11, 1912 | 7 | Rhesus 186–[5–11] | Peritoneum | 0 | 0 | – | Do. |
| Rhesus 143 | Oct. 13, 1911 | 3.6 | Rhesus 158–[1–9] | Peritoneum and vein. | 0 | 0 | – | Filtered serum. |
|  | Nov. 11, 1911 | 2.5 | Rhesus 163–[2–8] | Vein | 7 | 8 | + | Defibrinated blood. |
| Rhesus 144 | Oct. 13, 1911 | 3 | Rhesus 158–[1–9] | Peritoneum and vein. | 0 | 0 | – | Filtered serum. |
|  | Nov. 11, 1911 | 2.5 | Rhesus 163–[2–8] | Vein | 6 | 9 | + | Defibrinated blood. |
| Rhesus 147 | Oct. 22, 1911 | 4.5 | Rhesus 141–[3–.] | Vein | 0 | 0 | – | Serum. |
|  | Nov. 20, 1911 | 2.5 | Rhesus 166–[3–12] | Vein and subcutaneous. | 7 | 8 | + | Defibrinated blood. |
| Rhesus 148 | Oct. 22, 1911 | 3 | Rhesus 141–[3–.] | Vein | 9 | 10 | + | Serum. |
|  | Nov. 27, 1911 | 3 | Case 3N [2] | Vein | 0 | 0 | – | Defibrinated blood. |
| Rhesus 194 | Jan. 20, 1912 | 6 | Rhesus 309–[2–7] | Peritoneum. | 0 | 0 | – | Filtered serum. |
|  | Feb. 23, 1912 | 6 | Rhesus 200, 203, 204 | Peritoneum. | 0 | 0 | – | Defibrinated blood. |
|  | Mar. 30, 1912 | 3 | Rhesus 230, 233 | Peritoneum. | 6 | 10 | + | Do. |
| Rhesus 221 | Mar. 6, 1912 | 6 | Rhesus 210, 213 | Peritoneum. | 0 | 0 | – | Serum. |
|  | Mar. 30, 1912 | 3 | Rhesus 232, 233 | Peritoneum. | 0 | 0 | – | Defibrinated blood. |
|  | Apr. 20, 1912 | 3 | Rhesus 225 | Peritoneum. | 0 | 0 | – | Do. |
|  | May 4, 1912 | 3 | Rhesus 229 | Peritoneum. | 0 | 0 | – | Do. |
|  | May 9, 1912 | 3.5 | G. P. 70, 71, 72, 73 | Peritoneum. | 0 | 0 | – | Do. |
|  | May 31, 1912 | 8 | Rhesus 258 | Peritoneum. | 0 | 0 | – | Do. |
| Rhesus 223 | Mar. 6, 1912 | 6 | Rhesus 210, 213 | Peritoneum. | 0 | 0 | – | Filtered serum. |
|  | Mar. 30, 1912 | 3 | Rhesus 232, 233 | Peritoneum. | 8 | 9 | + | Defibrinated blood. |
| Rhesus 224 | Mar. 6, 1912 | 6 | Rhesus 210, 213 | Peritoneum. | 0 | 0 | – | Filtered serum. |
|  | Mar. 30, 1912 | 3 | Rhesus 232, 233 | Peritoneum. | 8 | 9 | + | Defibrinated blood. |

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[1] The first number in brackets indicates the day of disease and the second, when given, the duration of illness of the animal: so that under Rhesus No. 141 we should read "given 3.5 c. c. blood from Rhesus No. 158 on the first day of a fever lasting 9 days."

[2] M=Mexican strain: N=New York strain.

Seven monkeys received a primary inoculation of filtered serum; none of the seven responded to this initial inoculation; when subjected to an immunity test, however, all but two (Nos. 115*a* and 194) responded. In other words, two of the seven failed to react to the first injection of virulent material. Of these two, one, No. 194, responded to the second immunity test, and the other, No. 115*a*, to the third.

If now we combine the results summarized in the foregoing tables, we find that of 58 animals 13, or 22.5 per cent failed to react (did not become infected) after one injection of virulent blood or blood serum; 5, or 8.6 per cent, failed to react after two, and at least 2, or 3.5 per cent, after at least three injections. It is evident, then, that a very large proportion (22.5 per cent) of (rhesus) monkeys possess at least a transient natural immunity, and it seems reasonable to consider that in about 3.5 per cent of animals the resistance noted is sufficiently persistent to be regarded as a permanent natural immunity.

It may perhaps be objected that this is only an apparent immunity; that the resistance is simply due to a virus of low or varying virulence, to the smallness of the dose employed, to the site of inoculation, or to the size or age of the animal.

TABLE No. 3.—*Primary inoculation with human typhus blood.*

| Monkey No. | Date. | C. c. inoculated. | Source. | Site. | Incubation period (days). | Duration of disease (days). | Result. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| Rhesus 149. | Sept. 20, 1911 | 3 | Case 1N [1] | Peritoneum | 0 | 0 | − | |
| | Oct. 22, 1911 | 3.5 | Rhesus 141-[3-?][2] | Peritoneum and subcutaneous. | 8 | 29 | [3]+ | Relapse. |
| Rhesus 150. | Sept. 20, 1911 | 3 | Case 1N | Peritoneum | 10 | 13 | [3]+ | Do. |
| Rhesus 151. | Sept. 21, 1911 | 6 | do | Peritoneum | 0 | 0 | (4) | Cachexia. |
| Rhesus 152. | do | 6.5 | do | Peritoneum | 0 | 0 | (4) | Peritonitis. |
| Rhesus 160. | Oct. 29, 1911 | 3.5 | Case 2N | Vein | 0 | 0 | (5) | |
| Rhesus 161. | do | 4 | do | Peritoneum | 0 | 0 | − | |
| | Nov. 29, 1911 | 2.5 | Rhesus 170-[4-9] | Vein | 7 | 15 | + | |
| | Dec. 29, 1911 | 6 | Case 35M [1] | Peritoneum | 0 | 0 | − | |
| | Jan. 1, 1912 | 4 | Case 39M | | | | | |
| Rhesus 162. | Oct. 29, 1911 | 6 c. c. washed corpus. | Case 2N | Peritoneum and subcutaneous. | | | (6) | |
| | Nov. 29, 1911 | 2.5 | Rhesus 170-[4-9] | Vein | 7 | 12 | + | |
| | Dec. 29, 1911 | 6 | Case 35M | Peritoneum | 0 | 0 | − | |
| | Jan. 1, 1912 | 4 | Case 39M | | | | | |
| Rhesus 172. | Nov. 27, 1911 | 3 | Case 3N | Vein | | | − | |
| | Jan. 2, 1912 | 3 | Rhesus 171-[2-9] | Vein | | | (5) | |
| Rhesus 173. | Nov. 27, 1911 | 2.5 | Case 3M | Vein | 0 | 0 | − | |
| | Jan. 2, 1912 | 3 | Rhesus 171-[2-9] | Vein | 10 | 6 | + | |
| Rhesus 235. | Mar. 26, 1912 | 10 | Case 4N | Peritoneum | 0 | 0 | − | Tuberculosis. |

[1] M=Mexican strain; N=New York strain.

[2] The first number in brackets indicates the day of disease and the second, when given, the duration of illness of the animal; so that under Rhesus No. 161 we should read "given 2.5 c. c. blood from Rhesus No. 170 on the fourth day of a fever lasting 9 days."

[3] Died.

[4] Premature death.

[5] Tuberculosis.

[6] Pseudo reaction.

TABLE No. 3.—*Primary inoculation with human typhus blood*—Continued.

| Monkey No. | Date. | C. c. inoculated. | Source. | Site. | Incubation period (days). | Duration of disease (days). | Result. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| Rhesus 236. | Mar. 26, 1912 | 10 | Case 4N | Peritoneum | 0 | 0 | − | |
| | May 4, 1912 | 3 | Rhesus 229-[6-7] | Peritoneum | 0 | 0 | − | |
| Rhesus 301. | Nov. 9, 1911 | 7.5 | Case 1M | Peritoneum and subcutaneous. | ...... | .... | .... | Died (pneumonia). |
| Rhesus 307. | Nov. 19, 1911 | 3 | Case 9M | Peritoneum | ...... | .... | .... | Do. |
| Rhesus 315. | Dec. 2, 1911 | 2.5 | Case 16M | Vein | ...... | .... | − | |
| | Dec. 22, 1911 | 5 | Case 26M-[12-18] | Peritoneum | 8 | 8 | + | |
| | Feb. 1, 1912 | 2.5 | Rhesus 184-[4-14] | Vein | 0 | 0 | − | |
| Rhesus 316. | Dec. 2, 1911 | 2.5 | Case 16M | Vein | 0 | 0 | − | |
| | Dec. 22, 1911 | 4.5 | Case 26M | Peritoneum | 8 | 3 | + | Abortive. |
| | Feb. 1, 1912 | 2.5 | Rhesus 184-[4-14] | Vein | 0 | 0 | − | |
| | Feb. 23, 1912 | 6 | Rhesus 200, 203, 204. | Peritoneum | 0 | 0 | − | |
| Rhesus 317. | Dec. 8, 1911 | 3.5 | Case 19M | Peritoneum | 15 | 4 | + | |
| | Jan. 10, 1912 | 3 | Rhesus 187-[2-16] | Vein | 0 | 0 | − | |
| Rhesus 318. | Dec. 8, 1911 | 3.5 | Case 19M-[6-14] | Peritoneum | 7 | 10 | + | |
| | Jan. 10, 1912 | 3 | Rhesus 187-[2-6] | Vein | 0 | 0 | − | |
| Rhesus 323. | Dec. 29, 1911 | 6 | Case 35M | Peritoneum | ...... | .... | .... | Tuberculosis. |
| | Jan. 1, 1912 | 4 | Case 39M | | | | | |
| Rhesus 324. | Dec. 29, 1911 | 6 | Case 35M | Peritoneum | 8 to 5 | 10 | + | |
| | Jan. 1, 1912 | 4 | Case 38M | | | | | |
| Rhesus 325. | Dec. 30, 1911 | 6 | Case 38M-[11-15] | Peritoneum | 11 to 9 | 3 | + | Abortive. |
| | Jan. 1, 1912 | 4 | Case 39M | | | | | |
| | Feb. 1, 1912 | 2.5 | Rhesus 184-[4-14] | Vein | 0 | 0 | − | |
| Rhesus 326. | Dec. 30, 1911 | 6 | Case 38M | Peritoneum | 11 to 9 | 3 | + | Do. |
| | Jan. 1, 1912 | 4 | Case 39M | | | | | |
| | Feb. 1, 1912 | 2.5 | Rhesus 184-[4-14] | Vein | 0 | 0 | − | |
| | Mar. 6, 1912 | 6 | Rhesus 213, 212 | Peritoneum | 0 | 0 | − | |

It is readily conceivable that different strains of typhus virus may differ markedly in virulence. Analogies readily suggest themselves, and the results of our inoculations with virus from human sources appear to furnish some experimental support for such a view. In Table No. 3 we summarize our inoculations with blood from human cases of typhus. It will be seen that in only a small proportion, whether of the New York or the Mexican cases, were we successful. While, as we say, it is not improbable that in these cases viruses of differing virulence may have entered as an element, we do not believe that this factor enters into the results of the series of inoculations above considered; for the inoculations in question were made with a single strain, which we have successfully propagated through some 23 monkey generations, throughout which the virus, so far as we are able to discern, has maintained its original degree of virulence. The suggestion that age may enter as a factor in susceptibility has been advanced, because it is held by some that children are less susceptible than adults. As a matter of fact, there is no good evidence to show that children are really less susceptible to typhus than adults. It is probably true that the manifestations of typhus in a child are not in all respects like those in the adult, that is they are not "typical," but this manifestly can not properly be regarded as indicating a difference in the degree of susceptibility. That this and the other objections cited are not valid, however, may be clearly inferred from the fact, first, that of two monkeys of substantially the same size and vigor, both inoculated at the same time, by the same route but with different quantities of the same virus, the animal receiving the larger dose (by 50 per cent) failed to react (see rhesus Nos. 147 and 148, Table 2), and, second, as has already been noted above, an animal may react after the second or the third inoculation, although the dose may be very much smaller than that given in the immediately preceding ineffective inoculation.

The recognition of the existence of a more or less marked transient (or permanent) natural immunity in monkeys is of great practical importance in all work on typhus. Great caution and conservatism must be observed in the interpretation of experimental results, especially negative results. We believe, too, that it may have a much broader significance and application. It will be recalled, for instance, that Metchnikoff and Besredka (December, 1911, p. 866), in their studies on antityphoid vaccination, conclude that the ingestion of bacillus paratyphoid-B may vaccinate against true typhoid. This conclusion is based on the observation that in one of two experiments a chimpanzee that had previously been infected experimentally with paratyphoid-B was subsequently found refractory to infection with typhoid. Now, while the results of future work may reenforce this conclusion, the thought readily suggests itself that an occasional chimpanzee may be met with that, though susceptible to paratyphoid-B, may be transiently or permanently resistant to infection with typhoid. Indeed, these authors themselves, in a previous communication (March, 1911, p. 200), report that they met with one failure in 16 experiments to infect anthropoids. We believe, therefore, that their conclusion is to some extent at least, if not altogether, invalidated.

In a discussion of the susceptibility of the monkey it is essential to have a clear understanding of what one is to consider as a typhus reaction in this animal. Following an inoculation with virulent material the monkey continues for a period varying from 5 to 24 days as if nothing had happened. As may be seen from Table No. 4, the incubation period in about 90 per cent of cases varies between 6 and 10 days.

TABLE NO. 4.

| Incubation period. | Number of monkeys. | Incubation period. | Number of monkeys. |
|---|---|---|---|
| 5 days | 1 | 11 days | 4 |
| 6 days | 12 | 14 days | 4 |
| 7 days | 20 | 15 days | 1 |
| 8 days | 16 | 24 days | 1 |
| 9 days | 28 | | |
| 10 days | 16 | Total | 103 |

At the end of this period the temperature of the susceptible animal rises fairly rapidly as a rule, sometimes gradually, or at times very abruptly. The fever reaches its fastigium in 36 to 48, or 72 hours; it then continues for a variable period of 1 or 2 to 5 or more days, then defervesces. The defervescence, like the invasion, is variable; although usually gradual, it is frequently rapid or even critical. In brief, the course of the fever in the monkey is essentially like that of the fever in man.

The fever may be accompanied by loss of appetite, thirst, a ruffling of the fur, and a drooping posture; very commonly, however, even with a well-defined febrile reaction, the animal except for some slight listlessness shows hardly any outward manifestations. In other words, the fever is the only definite clinical index of a typhus reaction. As may be seen from Table No. 5, in about 76 per cent of the cases the fever varies in duration between 6 and 10 days. At the termination of the fever there is almost always manifest some degree of emaciation. Occasionally after the temperature has been normal for three or four days or a week it may go up a second time; such a relapse may last five or seven or more days and end in recovery or death. We have met with febrile recrudescence or relapse in 4 of 103 cases.

TABLE NO. 5.

| Duration of illness. | Number of monkeys. | Duration of illness. | Number of monkeys. |
|---|---|---|---|
| 3 days | 2 | 11 days | 9 |
| 4 days | 1 | 12 days | 6 |
| 5 days | 1 | 13 days | 3 |
| 6 days | 5 | 14 days | 2 |
| 7 days | 9 | 15 days | 1 |
| 8 days | 24 | 29 days | 1 |
| 9 days | 21 | | |
| 10 days | 17 | Total | 102 |

Although in appearance a mild disease, we have thus far had 4 deaths in a total of 103 cases of typhus in the monkey. This total of cases includes 10 induced with the Mexican virus. Segregating these, we have 4 deaths in 93 cases of the disease induced with the New York strain (Brill's disease), a mortality notably higher than that reported by Brill in the human subject.

TABLE No. 6.—*Results of immunity tests in animals that had previously presented a marked reaction.*

| Monkey No. | Date. | C. c. inoculated. | Source. | Site. | Incubation period (days). | Duration of disease (days). | Result. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| Rhesus 95 | Nov. 23, 1911 | 3 | Rhesus 306 | Vein | .... | .... | — | |
| | Dec. 8, 1911 | 3.5 | Case 19M[1] | Peritoneum | .... | .... | — | |
| Rhesus 127. | Dec. 30, 1911 | 6 | Case 38M | do | .... | .... | — | |
| | Jan. 1, 1912 | 4 | Case 39M | do | .... | .... | — | |
| Rhesus 133. | Nov. 23, 1911 | 3 | Rhesus 306 | Vein | .... | .... | — | |
| | Dec. 8, 1911 | 3.5 | Case 19M | Peritoneum | .... | .... | — | |
| Rhesus 142. | Nov. 29, 1911 | 5 | Rhesus 170 | Vein | .... | .... | — | |
| | Dec. 12, 1911 | 4 | Rhesus 161 | do | .... | .... | — | |
| | Jan. 11, 1912 | 7 | Rhesus 186 | Peritoneum | .... | .... | — | |
| Rhesus 158. | Nov. 9, 1911 | 5 | Case 1M | Vein and subcutaneous. | .... | .... | — | |
| | Dec. 2, 1911 | 2.5 | Case 16M | Vein | .... | .... | — | |
| | Dec. 22, 1911 | 4.5 | Case 26M | Peritoneum | .... | .... | — | |
| Rhesus 188. | Mar. 30, 1912 | 3 | Rhesus 234 | do | .... | .... | — | |
| Rhesus 210. | do | 3 | do | do | .... | .... | — | |
| Rhesus 306. | Dec. 29, 1911 | 6 | Case 35M | do | .... | .... | — | |
| | Jan. 1, 1912 | 4 | Case 39M | do | .... | .... | — | |
| | Feb. 1, 1912 | 2.5 | Rhesus 184 | Vein | .... | .... | — | |
| Rhesus 315. | do | 2.5 | do | do | .... | .... | — | |
| Rhesus 316. | do | 2.5 | do | do | .... | .... | — | |
| | Feb. 23, 1912 | 6 | Rhesus 200, 203, 204 | Peritoneum | .... | .... | — | |
| Rhesus 317. | Jan. 10, 1912 | 3 | Rhesus 187 | Vein | .... | .... | — | |
| Rhesus 318. | do | 3 | do | do | .... | .... | — | |
| Rhesus 500. | Nov. 9, 1911 | 4 | Case 1M | do | .... | .... | — | |
| | Dec. 2, 1911 | 2.5 | Case 16M | do | .... | .... | — | |
| | Jan. 10, 1912 | 3 | Rhesus 187 | do | .... | .... | — | |
| | Mar. 6, 1912 | 6 | Rhesus 213, 312 | Peritoneum | .... | .... | — | |

[1] M=Mexican.

Various types of the fever curve in the monkey have been published in our previous contributions. Additional illustrations appear in this paper.

An animal that has presented a reaction such as above described is immune from subsequent infection. In Table No. 6 we present the results of immunity tests in monkeys that had presented typical primary reactions. It will be seen that in no instance in our experience has such an animal responded to a subsequent immunity test. Such an immunity may last a long time, as is shown by the following: "Adela," a female rhesus (No. 500), was originally infected by an intraperitoneal injection of 6 c. c. of defibrinated blood from a patient with (Mexican) typhus on January 11, 1910. This animal developed a marked typhus (see p. 14), terminating on January 29–30, 1910. Between November 9, 1911, and March 6, 1912, this monkey was given four inoculations of typhus blood, all of which it resisted absolutely, showing that it was still immune after over two years. (See rhesus 500, Table No. 6.)

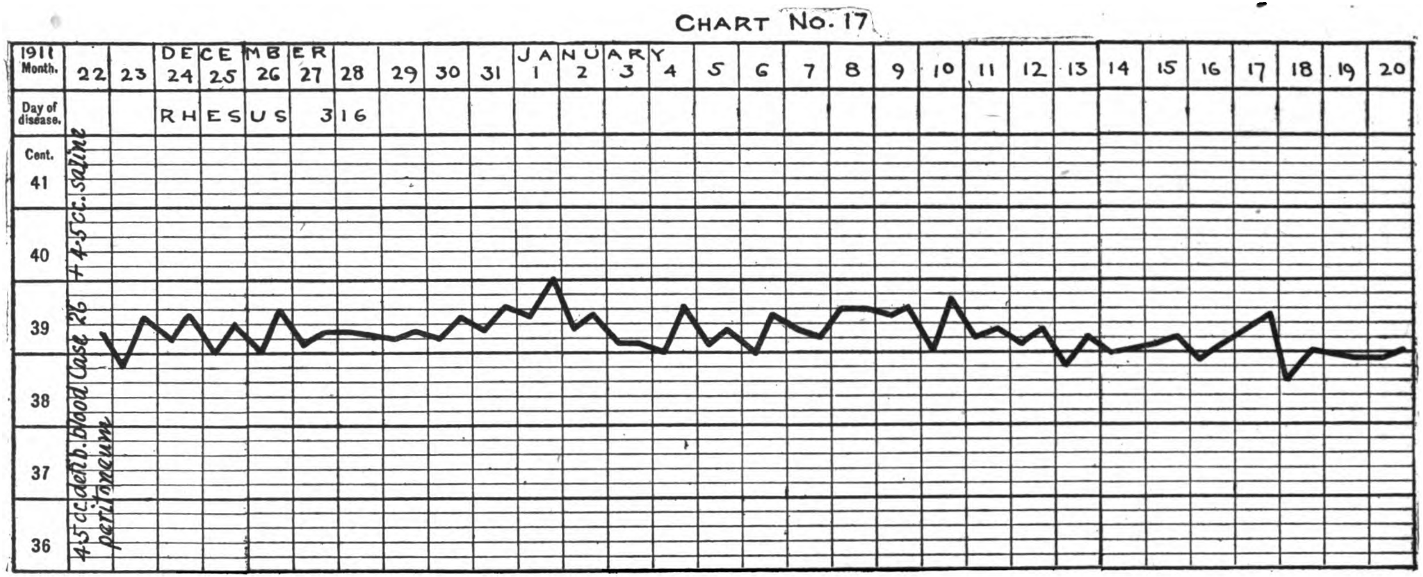

**CHART NO. 17.**—Temperature curve of rhesus No. 316, showing "abortive" reaction, with its acme on January 1.

Ordinarily, therefore, a well-marked febrile reaction may be interpreted as typhus without subjecting the animal to an immunity test for confirmation. When, however, the fever is slight or its course atypical—that is, when we have what may be designated as an "abortive" fever—this can not be construed as a typhus reaction unless the immunity test proves the animal resistant to infection, and even then not without some reserve. For examples of what we have considered "abortive" reactions the reader is referred to charts Nos. 17, 18, and 19. The perturbations in the temperature in the case of rhesus No. 316 and in that of No. 326 (charts Nos. 17 and 19) are clearly appreciable, but very slight; in the case of No. 325 it is better marked, but of short duration.

It may be of interest to note that monkeys Nos. 325 and 326 were inoculated with large doses of blood from two very severe cases of (Mexican) typhus. The extreme mildness of the reaction to this inoculation would suggest that there is no necessary relationship between the gravity of the case furnishing the virus and the severity of the reaction in the monkey induced by the inoculation. Rhesus No. 316 was later subjected to two immunity tests, No. 325 to one, and No. 326 to two, without any response. (See Table No. 6.)

Should the immunity test in such cases show that resistance has not been conferred, a diagnosis of previous typhus is not permissible. Chart No. 20 shows the temperature curve of monkey No. 162. It presents an atypical fever of short duration, which might be construed as an "abortive" typhus. But an attempt at passage with blood drawn at the height of this fever resulted negatively, and when later subjected to an immunity test rhesus No. 162 promptly developed a severe typhus reaction (chart No. 21), which conferred resistance to a subsequent inoculation with virulent typhus blood. We therefore consider that the short atypical fever shown by this monkey (chart No. 20) was not typhus.

It might be argued that this was an instance of an abortive attack that had failed to confer immunity. This possibility must of course be conceded; but inasmuch as in the present state of our knowledge we have no way of judging the nature of a fever in the monkey other than by the result of the immunity test, we do not consider it permissible, in view of the result of this test, to interpret the fever in monkey No. 162 as an abortive typhus.

## CONCLUSIONS.

1. Instances of a transient or permanent natural immunity of the monkey to typhus are not uncommon. In our experience 22.5 per cent of monkeys have failed to react to a first and 3.5 per cent to three or more successive inoculations with virulent blood or blood serum.

2. Repeated inoculations of virulent blood or blood serum, when not followed by a febrile reaction, confer no appreciable resistance. Failure of an animal to react to an immunity test can not, in the absence of a previous febrile reaction, be interpreted as indicating that it was protected (vaccinated) by the primary, apparently ineffective, inoculation.

CHART No. 18

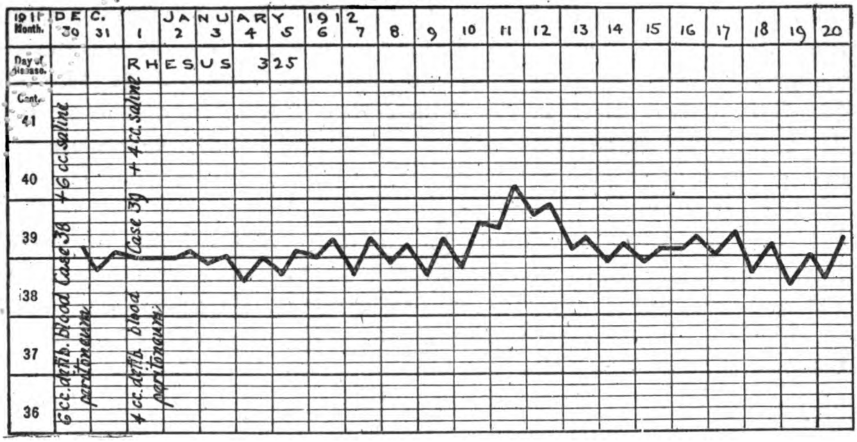

CHART No. 18.—Temperature curve of rhesus No. 325, showing "abortive" reaction.

3. A typical febrile reaction has, in our experience, always conferred complete protection to subsequent infection; in one instance for at least two years. Such a fever, therefore, under experimental conditions, justifies a diagnosis of typhus.

CHART No. 19

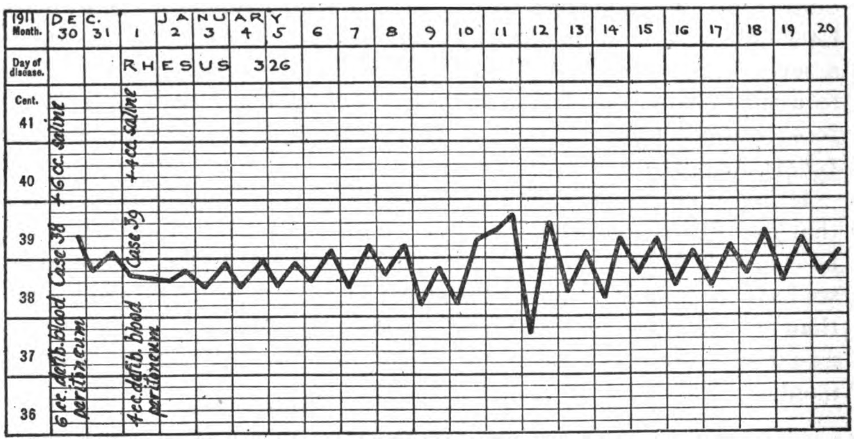

CHART No. 19.—Temperature curve of rhesus No. 326, showing "abortive" reaction.

4. An atypical or poorly defined (an "abortive") fever following inoculation can not be interpreted as typhus unless it is followed by resistance to subsequent immunity tests.

## SUSCEPTIBILITY OF THE GUINEA PIG AND THE RABBIT.

### GUINEA PIG.

We began the study of typhus in November, 1909, primarily with a view of determining its relationship to Rocky Mountain spotted fever. With this object in view we inoculated a series of guinea pigs with typhus fever blood and on December 10, 1909 (p. 10), reported that these inoculations had failed to produce either the signs or symptoms characteristic of Rocky Mountain spotted fever. Although our results appeared to be negative, we had noted, in some of the animals certain perturbations of temperature highly suggestive of a simple febrile reaction which made us suspect that the

CHART No. 20

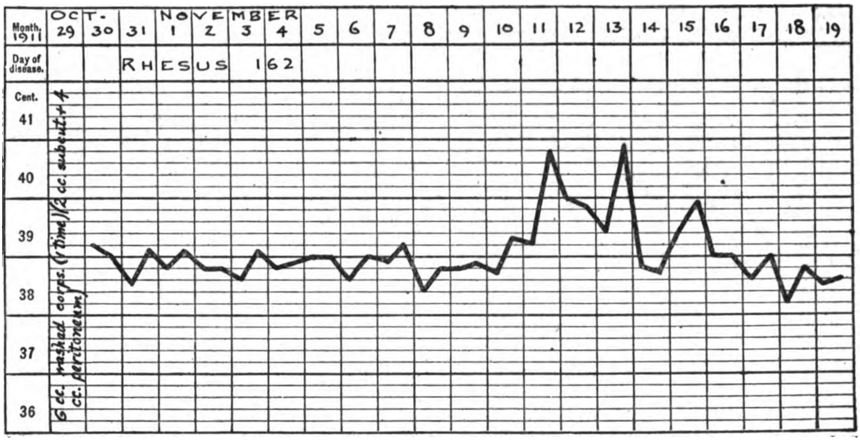

CHART No. 20.—Temperature curve of rhesus No. 162, showing pseudo reaction.

CHART No. 21

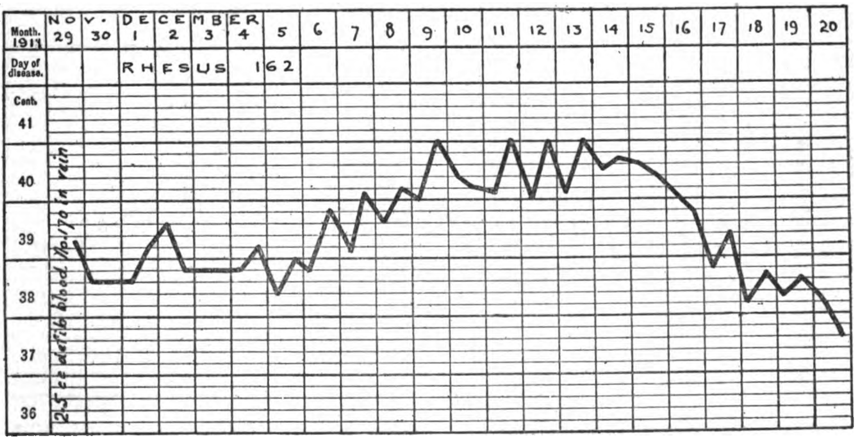

CHART No. 21.—Temperature curve of rhesus No. 162, showing marked reaction. Immunity not conferred by pseudo reaction shown in chart No. 20.

guinea pig might in some degree be susceptible to typhus. We therefore persisted in our attempts to infect the guinea pig with typhus. Unfortunately, during the progress of these experiments one of the authors, during the temporary absence of the other, was taken sick, resulting in the loss of the records of these later experiments.

CHART NO. 22

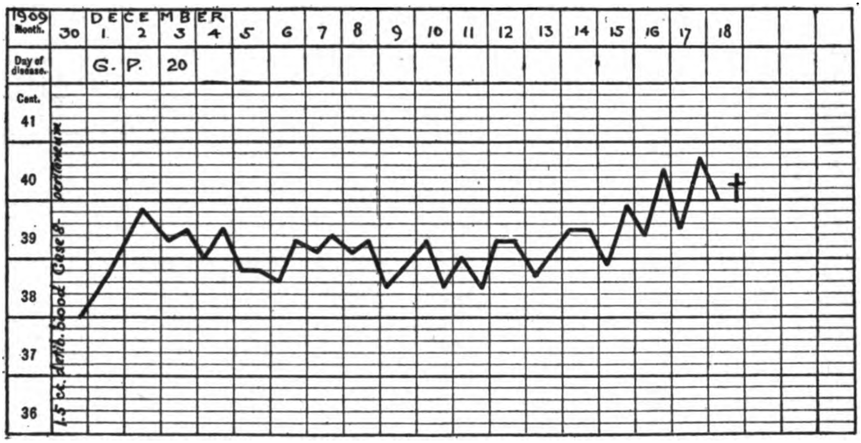

**Chart** No. 22.—Temperature curve of guinea pig No. 20, showing beginning of typhus reaction.

CHART NO. 23

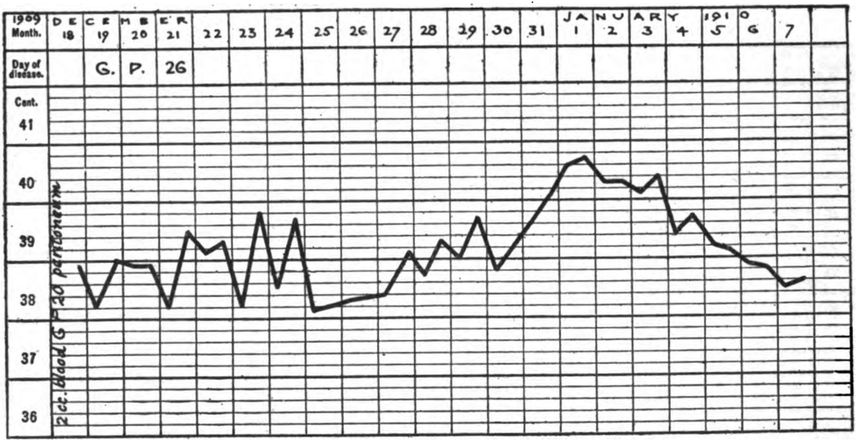

**Chart** No. 23.—Temperature curve of guinea pig No. 26, showing reaction following passage from pig No. 20.

Recently, however, in going over our old records and files we found amongst these some of the records which for two years we believed were lost. These records clearly show that we had successfully infected the guinea pig with Mexican typhus over two years ago. The credit, however, for being the first to demonstrate the susceptibility of the guinea pig to typhus undoubtedly belongs to Nicolle, Conseil, and Conor (June 6, 1911). These workers showed that the guinea pig reacts in essentially the same way to an inoculation with typhus blood as does the monkey. Their work was a few months later (Nov. 12, 1911) confirmed by Gaviño and Girard, who were able to propagate the virus by passage through 11 guinea pig generations.

Although preceded in this by both Nicolle, Conseil, and Conor, and by Gaviño and Girard, the publication of some of the results obtained by us over two years ago will not be without interest and value.

CHART NO. 24

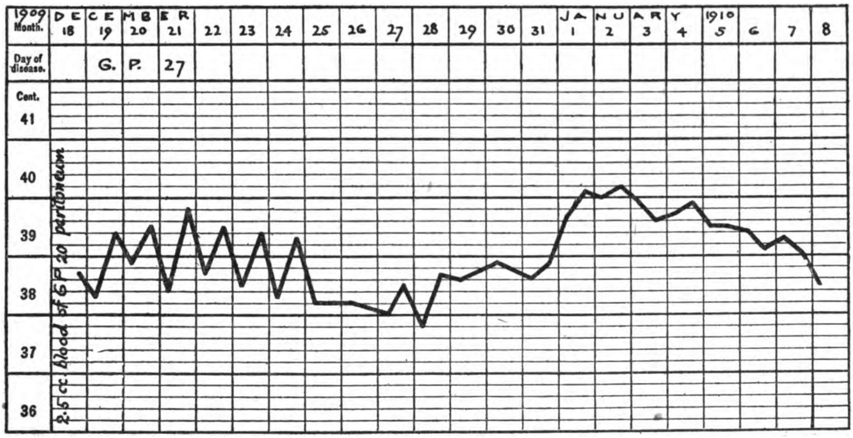

**Chart** No. 24.—Temperature curve of guinea pig No. 27, showing reaction following passage from pig No. 20.

*Experiment No. 1.*

On November 30, 1909, we inoculated two guinea pigs, Nos. 19 and 20, with 1.5 c. c. each of defibrinated blood of typhus fever case No. 8–M intraperitoneally. On the 15th, 16th, and 17th of December pig No. 19 presented a poorly defined but suggestive rise in temperature. In the case of guinea pig No. 20 (chart No. 22) the temperature showed a clearly marked progressive rise beginning December 15. On the eighteenth day after inoculation blood was drawn from the heart of this animal and after defibrination used for the inoculation of two fresh guinea pigs. As the result of the cardiac puncture, guinea pig No. 20 died. At the necropsy no gross lesions were discernible.

With the defibrinated blood of guinea pig No. 20 we inoculated pigs Nos. 26 and 27, each receiving 2 c. c. intraperitoneally. As may be seen in charts Nos. 23 and 24, both animals developed a clearly marked fever after an incubation of about 13 days in the former and 14 in the latter. Although we did not test the nature of this fever by the immunity test, there is no good reason to doubt that it was typhus. In our recent work with the New York virus we repeated these early experiments.

### *Experiment No. 2.*

On November 20, 1911, we inoculated two guinea pigs (A' and A) with virulent blood from rhesus No. 166, each receiving 0.5 c. c. intraperitoneally.

CHART No. 25

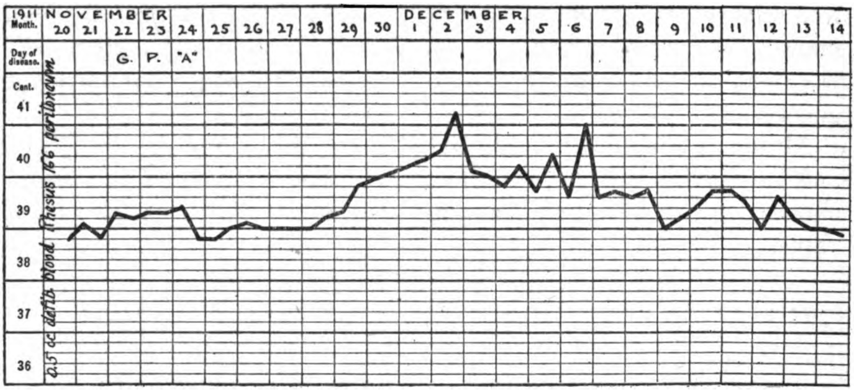

**Chart** No. 25.—Temperature curve of guinea pig A, showing reaction following inoculation with monkey typhus blood.

Following this inoculation, one of these pigs developed what was an abortive reaction; the other, however, presented a well-marked fever (chart No. 25) and furnished blood for passage to two fresh pigs, B and C. (See charts Nos. 26 and 27.) Each of the pigs B and C received 0.5 c. c. of defibrinated blood of guinea pig A, diluted with 1 c. c. of saline solution intraperitoneally.

CHART No. 26

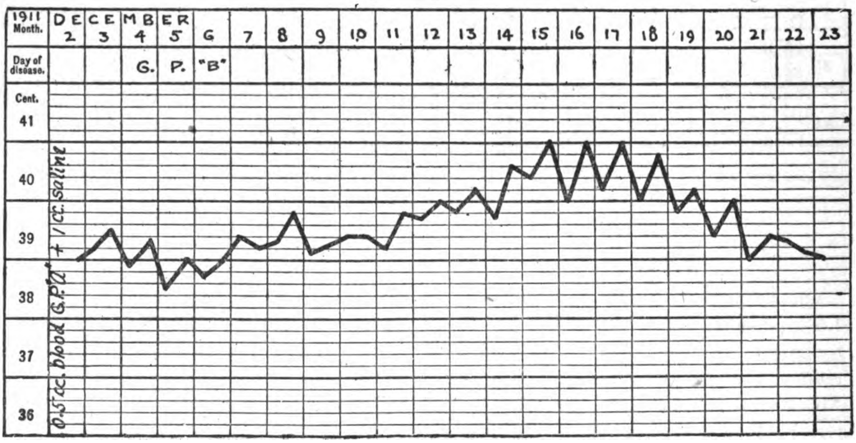

**Chart** No. 26.—Temperature curve of guinea pig B, showing reaction following passage from guinea pig A.

Both of these animals developed well-marked febrile reactions.

The pressure of our work on typhus in the monkey obliged us to temporarily suspend work with the guinea pig. On February 1, 1912, however, we again took this up.

### *Experiment No. 3.*

On February 1 we inoculated two guinea pigs (Nos. 30 and 32), each with 3 c. c of virulent blood from rhesus No. 184. Following this inoculation pig No. 32 developed an abortive febrile reaction; but pig No. 30 developed a well-marked fever and furnished the blood for passage. Since then we have successfully propagated this strain by passage in the guinea pig.[1]

CHART NO. 27

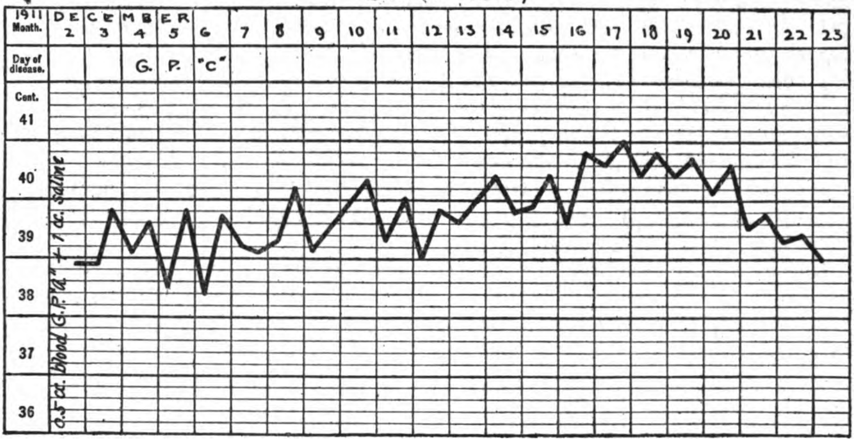

**Chart** No. 27.—Temperature curve of guinea pig C, showing reaction following passage from guinea pig A.

In order to satisfy ourselves that the fever induced in the guinea pig was indeed typhus we carried out the following experiment:

CHART NO. 28

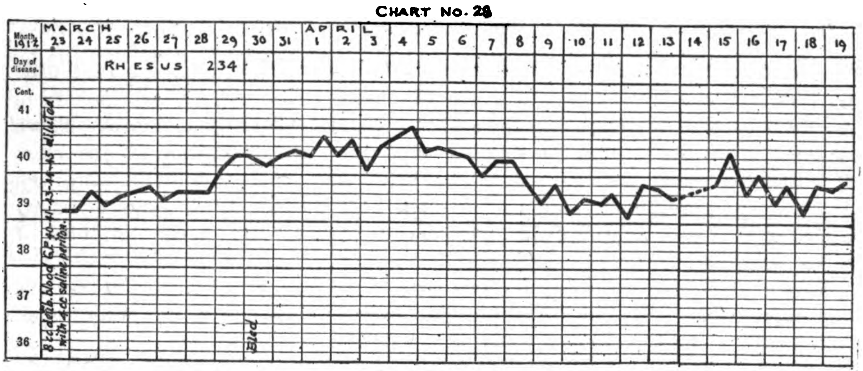

**Chart** No. 28.—Temperature curve of rhesus No. 234, showing reaction following passage from guinea pig.

### *Experiment No. 4.*

On March 23 ,1912, we inoculated rhesus No. 234 with 8 c. c. of defibrinated blood obtained from guinea pigs Nos. 40, 41, 43, 44, and 45, which represented the fourth guinea pig passage of this virus.

Following this inoculation rhesus No. 234 developed a well-marked febrile reaction (chart No. 28) after an incubation of six days.

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[1] On September 18 this virus was in the twenty-second guinea pig generation.

On the second day of this monkey's fever blood was drawn from its heart and, after defibrination, used for the following inoculations: Rhesus Nos. 237, 208, 220, 188, and 210, and Java No. 211, were each given 3 c. c. of the defibrinated blood diluted with 2 c. c. of saline solution intraperitoneally. Rhesus No. 237 was a fresh monkey; rhesus No. 208 had previously been subjected to an inoculation with dried typhus blood without having given a reaction; Java No. 211 and rhesus No. 220 had previously been subjected to inoculation with heated typhus blood. The inoculation of rhesus Nos. 208 and 220 and Java No. 211 with blood from rhesus No. 234 served therefore at once as an immunity test and (inasmuch as they were susceptible) as a control on rhesus Nos. 188 and 210, both of which had previously presented well-defined typhus reactions and were therefore typhus immunes.

*Result.*—Following these inoculations rhesus No. 237 (fresh) ran a high temperature, found to be due to tuberculosis. This monkey does not therefore enter into further consideration.

Rhesus Nos. 208 and 220, and Java No. 211, all three of which were regarded as susceptible to typhus, developed well-marked febrile reactions; but rhesus Nos. 188 and 210, both of which were typhus immunes, gave no appreciable indication of a reaction.

Conclusions.—It seems permissible to conclude, on the basis of the foregoing results, that some, if not all, of the guinea pigs (Nos. 40, 41, 43, 44, and 45) that furnished the virus for the inoculation of rhesus No. 234 were infected with typhus. By analogy it is perhaps permissible to assume that one attack of typhus in the guinea pig confers immunity from a second attack. But for the sake of completeness it seemed not undesirable to give this inference a direct experimental basis. Accordingly we performed the following experiment:

### *Experiment No. 5.*

On March 23, 1912, with some of the same defibrinated blood as that used for the inoculation of rhesus No. 234 (see experiment No. 4), obtained from guinea pigs Nos. 40, 41, 43, 44, and 45, we inoculated six guinea pigs. Three of these—Nos. 34, 35, and 36—had served for the second passage of this virus and had presented well-defined febrile reactions. The other three—Nos. 46, 47, and 48—were fresh. Each of the six pigs was given an intraperitoneal injection of 3 c. c. of the defibrinated blood diluted with an equal volume of saline solution.

*Result:* Following these inoculations none of the pigs, Nos. 34, 35, and 36, gave any appreciable indication of a reaction, whereas each one of the other three, Nos. 46, 47, and 48, developed well-marked febrile reactions.

Conclusion.—It seems permissible to conclude therefore that one attack of typhus in the guinea pig, as in the monkey, may confer (confers) immunity from a second attack.

## RABBIT.

In the course of our work on typhus in 1909–10 we made some attempts to infect the rabbit by inoculating with typhus fever blood. These were reported (June, 1910) in summary form, as follows:

*Rabbits.*—We inoculated three grown rabbits with varying quantities of some of the same blood as that used for some of the guinea pig inoculations. The inoculations were intraperitoneal in two and intravenous in one of these animals. During a period of observation of 30 days after inoculation these animals remained well and gained progressively in weight.

Since then Dreyer (1911), Gaviño and Girard (Nov. 12, 1911), and Nicolle and Conseil (May 25, 1912) have reported attempts to infect rabbits by injections of virulent typhus blood.

Dreyer (1911) attempted to infect two guinea pigs and two rabbits by subcutaneous injections of typhus blood from a sick monkey. The guinea pigs showed no sign of illness.

In the rabbits he noticed a fever that began in the evening of the day of inoculation and lasted four to six days. He is inclined to interpret this as an intoxication rather than as an infection with typhus.

Gaviño and Girard report two series of experiments. In the first they inoculated each of five rabbits with 3 c. c. of virulent defibrinated human blood and intraperitoneally. Following these inoculations the temperatures and weights of these animals were normal. In the second series they inoculated two rabbits, one being given 3. c. c. and the other 2 c. c. of virulent defibrinated human typhus blood intraperitoneally. The result in this series is also reported as having been entirely negative.

Nicolle and Conseil report that on May 8, 1911, they inoculated two rabbits of 1,800 and 1,900 grams intraperitoneally with 4.5 c. c. of virulent blood from a monkey in the fourth day of a grave typhus. The result was negative. The temperature was taken for 40 days.

On May 25, 17 days after the inoculation, they drew 6 c. c. of blood from the heart of one of these rabbits and injected it into the peritoneal cavity of a monkey. This monkey was not infected thereby.

In the course of the work that has grown out of our success in inoculating the monkey with New York typhus (" Brill's disease ") we renewed the attempts, first begun over two years ago, to infect the rabbit with this disease. Before presenting the results of these recent experiments we desire to report in detail the early experiments, the summary of which we cited above.

### INOCULATIONS OF 1909.

#### *Experiment No. 6.*

On November 23, 1909, we inoculated rabbit No. 1, weighing 2,350 grams, intraperitoneally with 6 c. c. of defibrinated blood from a typhus patient in the tenth day of a well-marked attack.

*Result:* During a period of 30 days following this inoculation this rabbit presented no recognizable indications of a reaction.

#### *Experiment No. 7.*

On November 30, 1909, rabbit No. 2, weighing 2,015 grams, was given an intravenous injection of 5 c. c. of defibrinated blood from a patient in the fifth day of a severe attack of typhus.

*Result:* This animal presented no recognizable evidence of a reaction during the 30 days following the inoculation.

### *Experiment No. 8.*

On December 2, 1909, rabbit No. 3, weighing 1,550 grams, was given an intraperitoneal injection of 8 c. c. of defibrinated blood from a patient in the sixth day of a severe attack of typhus.

*Result:* By referring to this animal's chart (chart No. 29) it will be noted that the temperature was slightly above its normal level for about six days beginning December 7, five days after the inoculation. Aside from this the animal appeared well and gained progressively in weight during the 30 days that it was kept under observation.

At the time that this experiment was performed we attached no significance to the slight rise in temperature. Nor are we at all certain of its significance at this time. However, in the light of our recent experience, about to be detailed, we think it possible that it may have been a very mild typhus reaction.

CHART NO. 29

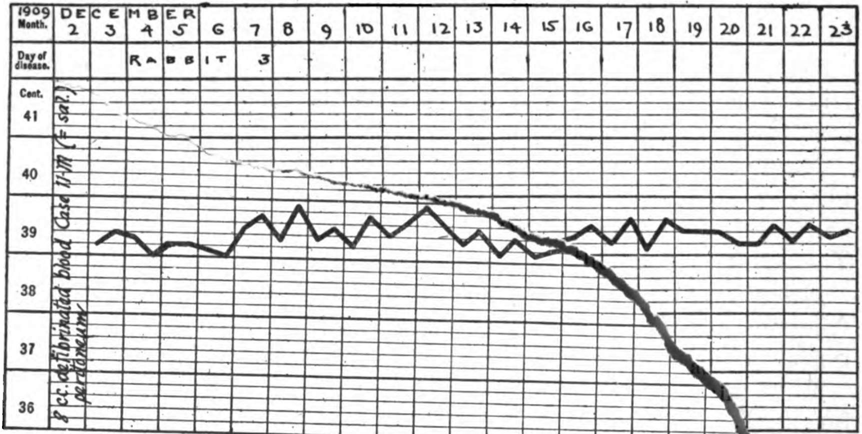

CHART No. 29.—Temperature curve of rabbit No. 3, following inoculation with Mexican typhus; possibly an abortive reaction December 7 to 12.

## INOCULATIONS OF 1912.

### *Experiment No. 9.*

On March 6, 1912, we inoculated three rabbits with virulent typhus monkey blood as follows: Rabbits Nos. 1 and 3 received 6 c. c. each and No. 2 received 5 c. c., intraperitoneally.

*Result:* During a period of observation of 40 days these animals presented no recognizable indication of a reaction.

### *Experiment No. 10.*

On March 23, 1912, with some of the same virulent defibrinated blood from guinea pigs Nos. 40, 41, 43, 44, and 45 as that used for the inoculati

CHART No. 30

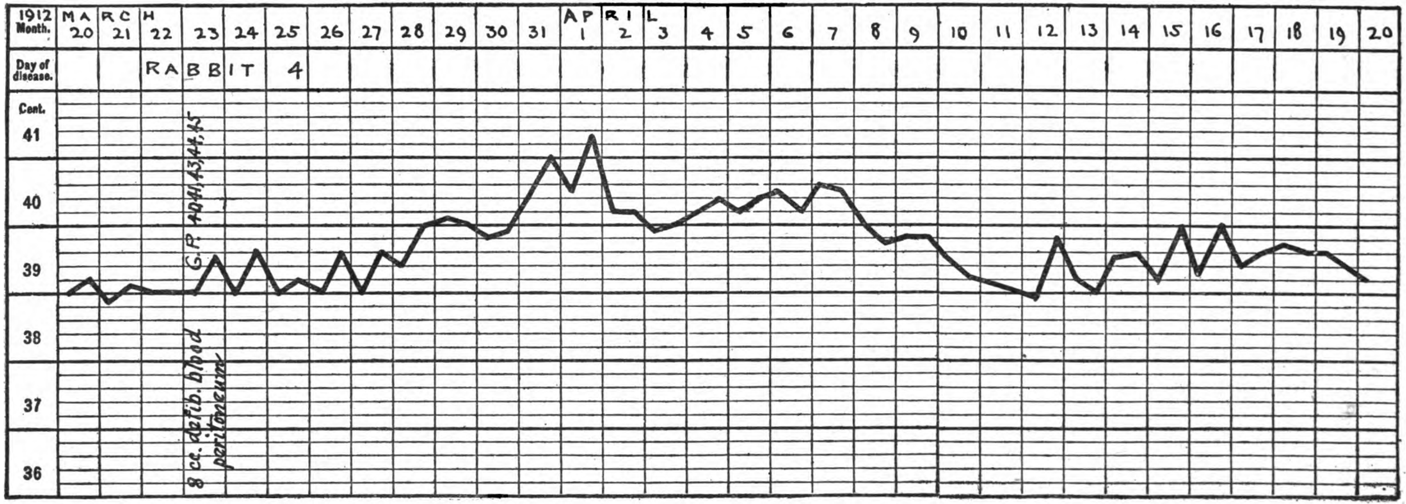

CHART No. 30.—Temperature curve of rabbit No. 4, showing reaction following inoculation with guinea pig typhus blood.

rhesus No. 234 (experiment No. 4) and pigs Nos. 34, 35, 36, 46, 47, and 48, we inoculated three rabbits, Nos. 4, 5, and 6, each receiving 8 c. c., intraperitoneally.

*Result:* After an incubation period of five days all three animals developed fever, most sharply marked in rabbit No. 5. As may be seen, by consulting charts Nos. 30, 31, and 32, the fever in rabbit No. 4 continued from March 28 to April 7 or 8; in rabbit No. 5 it continued from March 28 to about April 4; and in rabbit No. 6 from March 28 to April 5. Aside from the elevation in temperature, these animals presented no definite symptoms.

CHART NO. 31

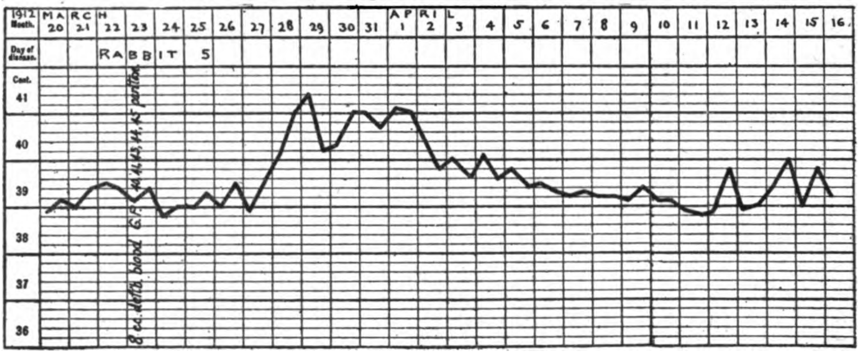

CHART No. 31.—Temperature curve of rabbit No. 5, showing reaction following inoculation with guinea pig typhus blood.

PASSAGE FROM RABBITS NOS. 4, 5, AND 6.—The early portion of the fever curve in these animals was probably modified in some degree by a bleeding to which each was subjected on March 29. Blood was drawn from the heart of each, defibrinated and mixed. With this pooled blood we inoculated five fresh rabbits, as follows: Nos. 7 and 10 received 8 c. c. diluted with 6 c. c. of saline solution, No. 9 received 6.5 c. c. diluted with 3 c. c. of saline solution, and No. 11 received 6 c. c. diluted with 3 c. c. saline solution, all intraperitoneally.

CHART NO. 32

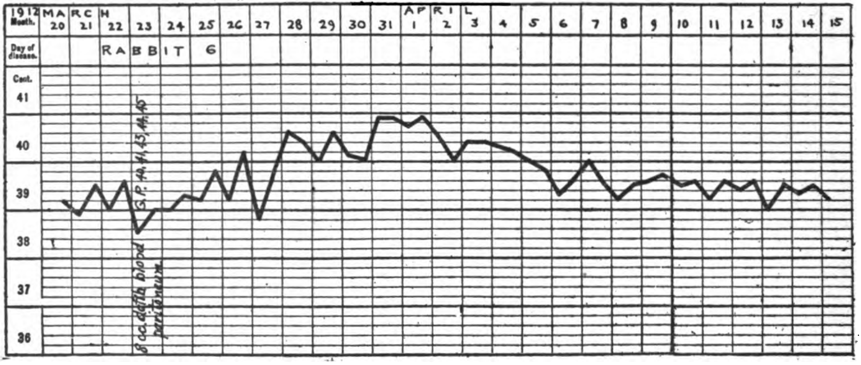

CHART No. 32.—Temperature curve of rabbit No. 6, showing reaction following inoculation with guinea pig typhus blood.

*Result:* Rabbit No. 7: Seven days after inoculation the temperature began to rise, reaching its maximum two days later. From its high point, 40.5°, the temperature dropped abruptly to 39.2°, but again rose slightly and continued

## CHART NO. 33

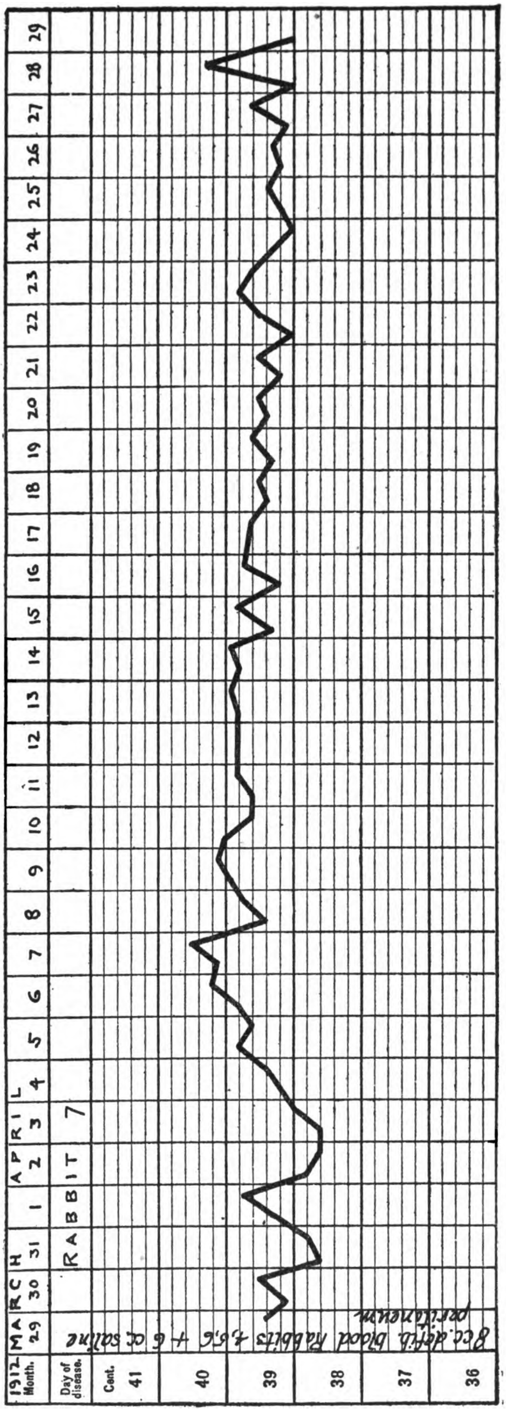

CHART No. 33.—Temperature curve of rabbit No. 7, showing reaction following passage from rabbits Nos. 4, 5, and 6.

at this slight elevation until April 14 or 15, when it may be considered as returning to its normal level (chart No. 33).

Rabbit No. 8: Six days after inoculation the temperature rose sharply to 41.2°; it remained up some 36 to 48 hours, during which time the animal presented no other symptoms (chart No. 34). In the morning of April 6—that is, 48 hours after it rose—this rabbit was found in apparent collapse, with a temperature of 37.6°; at 10.15 a. m. some blood was aspirated from its heart for passage. After the bleeding the rabbit seemed to revive somewhat, but died late in the afternoon. Unfortunately, through a misunderstanding, no necropsy was performed.

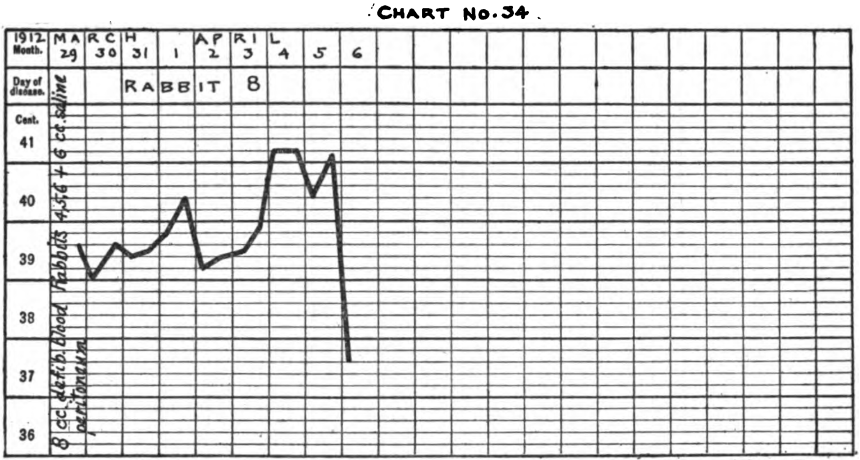

**Chart** No. 34.—Temperature curve of rabbit No. 8, showing reaction following passage from rabbits Nos. 4, 5, and 6.

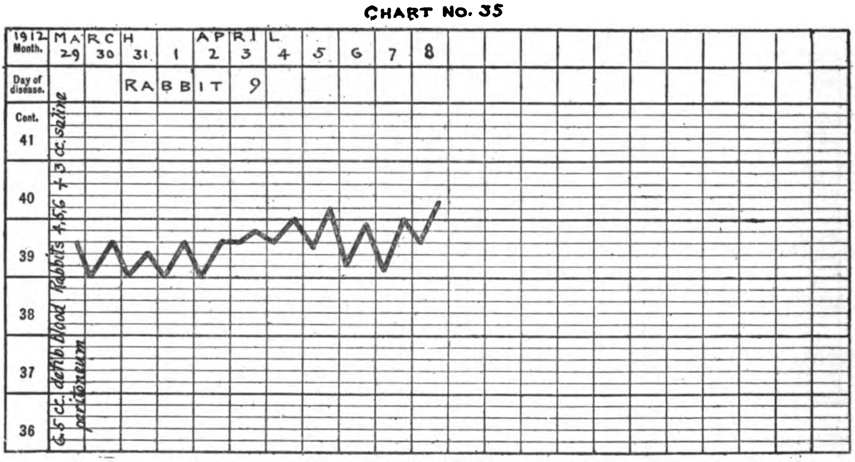

**Chart** No. 35.—Temperature curve of rabbit No. 9, showing reaction following passage from rabbits Nos. 4, 5, and 6.

Rabbit No. 9: Ten days after inoculation—that is, on April 8—the temperature of rabbit No. 9 was thought to be going up (chart No. 35). We therefore attempted to draw blood from the heart for passage. Unfortunately, in so doing the animal was killed, so that further observation was impossible. At necropsy no gross lesions were to be seen.

Rabbit No. 10: As in the case of rabbit No. 9, the temperature of rabbit No. 10 was also thought to be going up on April 8. At 1 p. m. on that day the temperature of this rabbit was 40.5°. At 1.20 p. m. we drew blood from the heart; but, as in the case of rabbit No. 9, the animal was unfortunately killed in so doing. At necropsy no gross lesions could be detected.

Rabbit No. 11: Seven days after the inoculation the temperature of No. 11 rose abruptly and remained up from April 5 until April 11, when it declined with equal abruptness. In the morning of April 13 this animal was found dead. At necropsy the lower lobe of the left lung was found to be pneumonic.

PASSAGE FROM RABBIT No. 8.—As stated above, blood was drawn from the heart of rabbit No. 8 on April 6. With this blood we inoculated three guinea pigs, Nos. 52, 53, and 54, each receiving 3 c. c. diluted with 2 c. c. of saline solution intraperitoneally.

*Result:* Guinea pig No. 52: Eleven days after inoculation this animal's temperature rose and may be regarded as remaining up about five days. In appearance this suggests a mild febrile reaction. When later subjected to an immunity test, however, this animal responded promptly and with a well-marked fever.

Guinea pig No. 53: This pig gave no recognizable evidence of a reaction. It was subjected to an immunity test with pig No. 52 on May 9, to which it responded with a mild febrile reaction. It had, therefore, been neither infected nor immunized by the blood inoculation from rabbit No. 8.

Guinea pig No. 54: This animal presented two febrile periods separated by intermission of about four days. The first period began three days after inoculation and its significance is not clear. The second period corresponds to the febrile period in pig No. 52 and would suggest a mild reaction to the inoculation. We think that this may have been the case, although passage with blood from this pig drawn on the last day of fever failed to infect any one of the three pigs inoculated (with 3 c. c. of whole blood each intraperitoneally).

PASSAGE FROM RABBITS NOS. 9 AND 10.—With the blood obtained from rabbits Nos. 9 and 10 on April 8, passage was attempted by inoculating rabbits Nos. 15, 16, and 17, each receiving 8 c. c. of the pooled defibrinated blood diluted with 2 c. c. of saline solution intraperitoneally.

As controls, monkeys Nos. 240 and 241 and guinea pigs Nos. 55, 56, and 57, were each given 3 c. c. diluted with 2 c. c. of saline solution intraperitoneally.

*Result:* Rabbit No. 15: Ten days after inoculation the temperature of this rabbit rose sharply, but at once dropped with equal abruptness. The temperature continued to fall and the animal two days later being practically moribund, was chloroformed. At necropsy no gross lesions were noted. Further passage with blood from this rabbit, aspirated from the heart just before being chloroformed, was without apparent result in two guinea pigs, each of which received 5 c. c. intraperitoneally.

Rabbits Nos. 16 and 17: Neither animal presented recognizable evidence of a reaction.

*Controls:* Neither the two monkeys (Nos. 240 and 241) nor the three guinea pigs, inoculated as controls, presented any recognizable evidence of a reaction. But in this connection it should be noted that, although rhesus Nos. 240 and 241 were fresh monkeys their susceptibility to typhus was apparently not high, for when this was later tested by inoculations of known virulent blood, rhesus No. 240 presented what may have been a mild abortive reaction, while No. 241 presented no appreciable evidence of a reaction.

INTERPRETATION.—It is evident from the foregoing that following an inoculation with virulent typhus guinea pig blood, rabbits Nos. 4, 5, and 6 simultaneously developed a fever after an incubation period of five days. Passage from these animals to five other rabbits was followed by the development of a more or less marked fever in all five. Excluding the animal that was found with pneumonic consolidation at necropsy, the fever in at least four of these five was probably of the same nature.

The results of further passage into guinea pigs, rabbits, and monkeys were poorly defined and unsatisfactory.

It is clear that the data presented may be interpreted in more than one way. We believe, however, that the most probable interpretation is that the fever in rabbits Nos. 4, 5, and 6 was typhus.

### *Experiment No. 11.*

On April 4, 1912, with defibrinated blood of rhesus No. 233, then in the seventh day of a marked experimental typhus, we inoculated three rabbits. Nos. 12 and 13 each receiving 6 c. c. diluted with 4 c. c. of saline solution, and No. 14, 5 c. c. diluted with 2 c. c. of saline solution, intraperitoneally.

*Result:* Rabbit No. 12: Fourteen days after inoculation this animal's temperature rose slightly and, but for a remission two days later, remained up for four days.

Rabbit No. 13: This animal's temperature appeared normally to be high, running at or slightly under 40°. On April 18—14 days after inoculation—it rose decidedly above its normal range and, remaining up, we, 2 days later, attempted passage with blood aspirated from the heart. The animal died as a consequence of the heart puncture. At necropsy a small subcutaneous abscess was found in the region of the right groin. This probably accounted for the abnormally high temperature presented from the first.

Rabbit No. 14: During a period of observation of 34 days this animal presented no evidence of a reaction.

PASSAGE FROM RABBIT No. 13.—With the blood obtained from rabbit No. 13 we inoculated three other rabbits, Nos. 18, 19, and 20, each receiving 8 c. c. diluted with 2 c. c. of saline solution intraperitoneally.

*Result:* Between April 27 and 30 rabbit No. 18 presented a slight perturbation in temperature that may perhaps represent a mild abortive fever. Otherwise, neither this nor the other two animals presented any indication of a reaction during a period of observation of 32 days.

INTERPRETATION.—Of the three rabbits inoculated with typhus monkey blood, one clearly presented a slight rise in temperature of about three days' duration after an incubation period of four days. This may have been, and we think probably was, an abortive typhus.

### *Experiment No. 12.*

On April 23, with defibrinated blood from rhesus No. 225 then in the eighth day of a severe attack of experimental typhus, we inoculated rabbits Nos. 21, 22, and 23 each with 8 c. c. diluted with 2 c. c. of saline intraperitoneally.

*Result:* During a period of observation of 30 days none of these animals gave any evidence of a reaction.

### *Experiment No. 13.*

On May 9, 1912, we inoculated rabbits Nos. 24 and 25 with virulent typhus guinea-pig blood, each rabbit receiving 8.5 c. c. intraperitoneally.

*Result:* During a period of observation of 30 days neither rabbit presented any recognizable indication of a reaction.

Conclusions.—It is evident from the results of the foregoing experiments, as interpreted by us, that some (a small proportion of) rabbits may be susceptible to infection with typhus.

## SPECIFICITY OF IMMUNITY.

In the course of our work the opportunity presented itself to test the resistance of a typhus-immune monkey to Rocky Mountain spotted fever and that of a spotted-fever immune animal to typhus, and also the resistance of measles-immune monkeys to typhus. Briefly stated, these tests were as follows:

### A. RESISTANCE OF TYPHUS-IMMUNE MONKEY TO ROCKY MOUNTAIN SPOTTED FEVER.

The following inoculation was very kindly made for us by our colleague Dr. McClintic:

On November 21, 1911, after a convalescence of 11 days from a well-marked experimental typhus, rhesus No. 155 was given an intraperitoneal injection of 1.5 c. c. of defibrinated blood from guinea pig No. 309, sick with spotted fever. Four days later rhesus No. 155 developed an attack of spotted fever, from which he died in 6 days. It is evident, therefore, that the attack of typhus had conferred on rhesus No. 155 no appreciable resistance to spotted fever.

Ricketts and Wilder report the same result from a simliar experiment.

### B. RESISTANCE OF ROCKY MOUNTAIN SPOTTED FEVER IMMUNE MONKEY TO TYPHUS FEVER.

On October 22, 1911, after 10 days' convalescence from a well-marked attack of spotted fever, rhesus No. 159 was inoculated with 3.5 c. c. of defibrinated typhus blood of rhesus No. 141, partly intravenously and partly intraperitoneally. To this inoculation rhesus No. 159 did not respond; but when reinoculated 29 days later with 2.5 c. c. of defibrinated typhus blood of rhesus No. 166 intravenously, monkey No. 159 responded sharply after an incubation period of 10 days.

The failure of monkey No. 159 to react to the first inoculation with virulent typhus blood might suggest that the previous attack of spotted fever had conferred on this animal temporary resistance to typhus; but when it is recalled that the normal monkey may behave in exactly the same way to a typhus inoculation it is evident that such an interpretation is not permissible. On the contrary, we believe that the attack of spotted fever had conferred no appreciable resistance to infection with typhus.

### C. RESISTANCE OF MEASLES-IMMUNE MONKEY TO TYPHUS FEVER.

Several of the animals used by us in our work on measles have been used by us in our typhus work and have been successfully inoculated with typhus, displaying no more resistance than perfectly fresh monkeys.

It appears, therefore, that an attack of measles in the monkey confers no appreciable resistance to subsequent infection with typhus, and, as Nicolle and Conseil (Dec. 26, 1911) report the successful inoculation of a typhus-immune monkey with measles, it would seem that an attack of typhus confers no appreciable resistance to measles.

Conclusions.—From the foregoing it seems reasonable to conclude that the immunity conferred by an attack of typhus is, so far as it has been tested, specific.

## PREVENTIVE ACTION OF IMMUNE SERUM.

The preventive power of typhus-immune serum was first studied by Nicolle and Conseil (September, 1910). These workers reported four experiments which may be summarized as follows:

### *Experiment No. 1.*

Bonnet monkey No. 57 was given a subcutaneous injection of 4 c. c. of a mixture of filtered serum, obtained from two patients in the 9th and 11th day of convalescence, respectively. Six hours later this animal was given an intraperitoneal injection of 4 c. c. of virulent typhus blood. Result: Negative, no febrile reaction.

### *Experiment No. 2.*

A *Macacus inuus*, No. 6, was given a subcutaneous injection of 2 c. c. of a mixture of filtered sera from three patients, one in the fourteenth and both of the others in the eleventh day of convalescence. Twenty-six hours later this animal was given an intraperitoneal inoculation of 5 c. c. of virulent typhus blood. Result: Entirely negative.

### *Experiment No. 3.*

In this experiment the inoculation (intraperitoneal) with virulent blood was made at the same time as that (subcutaneous) with serum from patients in the fourteenth, twentieth, and twenty-seventh days of convalescence. Of two monkeys one only was apparently protected by the serum.

### *Experiment No. 4.*

Bonnet monkey No. 50 was given a subcutaneous inoculation of 2 c. c. of serum from a chimpanzee that was three months convalescent and that a month before had been given an immunity test. Seventeen hours later this animal was given an intraperitoneal injection of 5 c. c. of virulent typhus blood. Result: This animal developed a typhus of medium severity after a somewhat prolonged incubation.

From the foregoing experiments Nicolle and Conseil conclude that the serum of convalescents and of recovered animals has a preventive action; that this action is complete with serum 9 and 11 days after the fall in temperature (experiments 1 and 2); that it is incomplete or uncertain with the sera obtained at a later period in man or chimpanzee (experiments 3 and 4).

Gaviño and Girard (1911) have also tested the preventive action of convalescent serum. They report a single experiment:

> Ten c. c. of serum from a guinea pig in the fourth day of convalescence was mixed with 6 c. c. of virulent blood. After a half hour's contact at laboratory temperature the serum and virus were subcutaneously inoculated into a monkey (*Cebus hypoleucus*). This monkey presented no appreciable reaction to the inoculation; from which they, rather prematurely we believe, conclude that this "demonstrates that the serum of an animal convalescent from typhus destroys the exanthematic virus in vitro."

It is in the nature of the problem that the evidence on which the preventive action of typhus-immune serum must be based is of a negative character. Negative evidence is convincing largely in proportion to its mass and this must be particularly the case when working with a species of varying individual susceptibility. For this reason it seemed desirable to repeat and extend the work of Nicolle and Conseil. We therefore report the following experiments:

### *Experiment No. 14.*

At 9.20 a. m., December 23, 1911, we injected subcutaneously rhesus No. 184 with 5 c. c. of blood serum obtained on the sixth day of convalescence from rhesus No. 129. One hour later—that is, at 10.30 a. m.—we gave monkey No. 184 an intravenous injection of 2.5 c. c. of virulent blood.

*Result:* During a period of observation of 28 days rhesus No. 184 presented no recognizable indications of a reaction. When subjected to an immunity test at the end of this period, rhesus No. 184 developed a marked reaction following a period of incubation of 9 days.

### *Experiment No. 15.*

At 1.10 p. m., January 20, we inoculated rhesus No. 193 with 3 c. c. of virulent defibrinated blood intravenously. One hour later—that is, at 2.10 p. m.—we gave this animal a subcutaneous injection of 5 c. c. of typhus-immune serum obtained on the thirtieth day of convalescence from rhesus No. 129 (see experiment No. 14).

*Result:* After an incubation of 10 days rhesus No. 193 developed a well-marked febrile reaction (chart No. 36). It may be noted in this connection that in the case of control rhesus No. 192 the incubation period was slightly longer—11 to 12 days—and the fever less marked (see charts Nos. 36 and 37). Mention is made of this because Nicolle and Conseil are inclined to explain a somewhat similar occurrence in a monkey that received immune serum as due to the influence of the serum. It is evident that the reacting body—the monkey—can not be disregarded as a factor, and a most important one, in the interpretation of such phenomena.

*Discussion.*—The immune serums used in the preceding two experiments were obtained from the same monkey, on the sixth day of convalescence in the first and the thirtieth in the second. While we can not be sure that infection of the monkey in the first experiment was prevented by the action of the serum, it is certain that in the second the serum neither prevented nor appreciably modified the infection (compare charts Nos. 36 and 37).

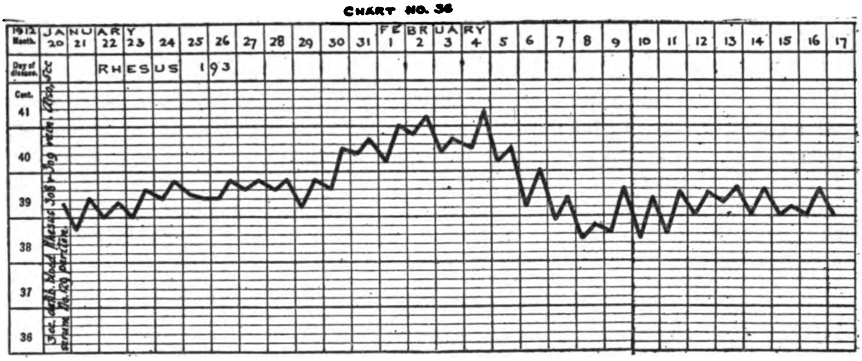

**Chart No. 36.**—Temperature curve of rhesus No 193, to show reaction indicating lack of protective action of immune serum obtained on thirteenth day of convalescence.

If, as seems probable, infection in the first one of these experiments was prevented by the immune serum, then the result of the immunity test would point either to a precise neutralization of the virus, or, if hyperneutralized, then passive immunity was not appreciable after 28 days.

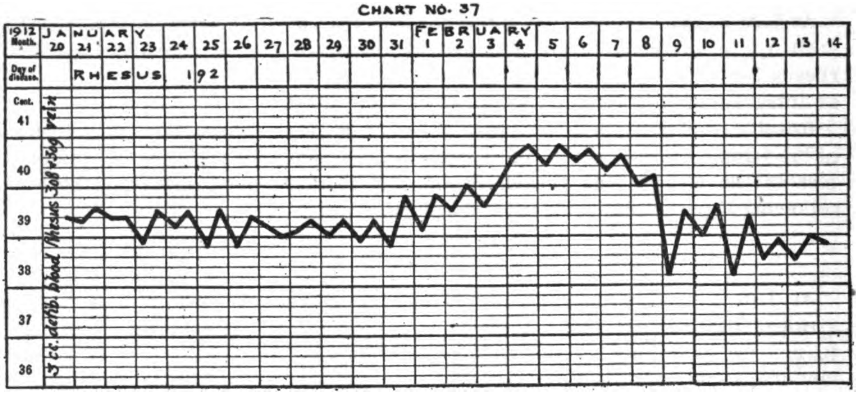

**Chart No. 37.**—Temperature curve of rhesus No. 192, control for No. 193 (chart No. 36), to show difference in incubation period and character of reaction.

### *Experiment No. 16.*

At 9.15 a. m., December 23, 1911, we gave rhesus No. 182 a subcutaneous injection of 5 c. c. of human blood serum obtained from case No. 1–M on the fourteenth or fifteenth day of convalescence. One hour later, or at 10.20 a. m., this monkey was given an intravenous injection of 2.5 c. c. of virulent blood.

*Result:* During a period of observation of 28 days this animal presented no indications of a reaction. When subjected to an immunity test at the end of this period rhesus No. 182 presented a well-marked febrile reaction after a period of incubation of 12 days.

This result points to a preventive action of the human immune serum. The serum was from the Mexican disease and by its action apparently prevented the infection of rhesus No. 182 with the New York (Brill's) disease; this may be regarded, therefore, as additional evidence of the identity of the Mexican and the New York disease.

As in the first of this group of experiments, either precise or hyperneutralization of the virus took place; in the latter event, such passive immunity as may have been conferred was not appreciable after 28 days.

### *Experiment No. 17.*

At 12.10 p. m., February 23, 1912, rhesus No. 214 was given an intraperitoneal injection of 6 c. c. of virulent defibrinated blood. About two hours later—that is, at 2.20 p. m.—this animal received a subcutaneous injection of 5 c. c. of monkey (No. 196) typhus-immune serum obtained on the eighth day of convalescence.

*Result:* During a period of observation of 29 days rhesus No. 214 presented no evidence of a reaction. Subjected to an immunity test on March 30, this monkey, after an incubation period of nine days, developed a severe febrile reaction.

This result would point to a preventive action of the immune serum when administered two hours after the inoculation of the virus.

As in two of the preceding experiments of this group, if, as seems probable, that infection was prevented by the immune serum, then either precise or hyperneutralization of the virus took place; in the latter event the immunity test showed that there was no appreciable passive immunty present after 36 days.

### *Experiment No. 18.*

At 12.12 p. m., February 23, 1912, rhesus No. 215 was given an intraperitoneal injection of 6 c. c. of virulent defibrinated blood. About 48 hours later—that is, at 11.55 a. m. of February 25—this animal was given a subcutaneous injection of 5 c. c. of monkey (No. 196) typhus-immune serum obtained on the eighth day of convalescence (see experiment No. 17).

*Result:* During a period of observation of 29 days this animal presented no evidence of a reaction. On March 30 rhesus No. 215 was subjected to an immunity test; 20 days later, not having in the meanwhile presented any evidence of a reaction, rhesus No. 215 was given a second immunity test. Ten days after this inoculation the temperature of the monkey rose a little and continued with rather marked oscillations (chart No. 38) for about nine days; this, though suggesting a mild febrile reaction, was not sufficiently well marked to permit of definite interpretation.

On the whole it would appear as if the immune serum, injected 48 hours after inoculation of the virus, had prevented infection. The failure to respond to two successive immunity tests would suggest that, although infection had been prevented, an immunity had been induced.

CHART No. 38

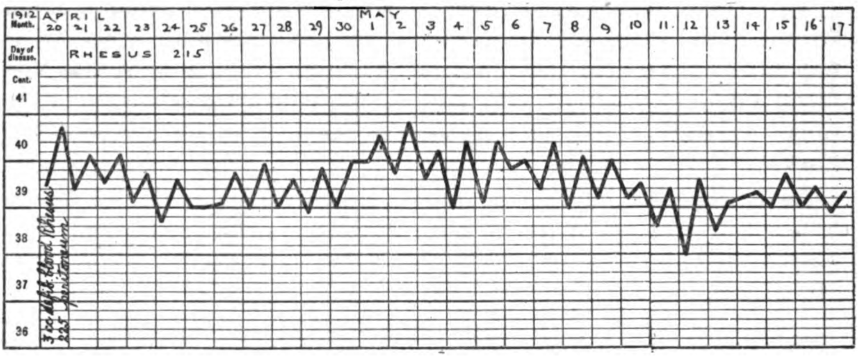

**Chart No. 38.**—Temperature curve of rhesus No. 215, after the second immunity test, showing a possible but doubtful reaction.

*Experiment No. 19.*

At 1.22 p. m., February 12, rhesus No. 205 was given an intraperitoneal injection of 3 c. c. of virulent defibrinated blood. About 48 hours later—that is, at 2 p. m. February 14, 1912—this animal was given a subcutaneous injection of 5 c. c. of monkey (No. 193) typhus-immune serum collected on the fifth day of convalescence.

CHART No. 39

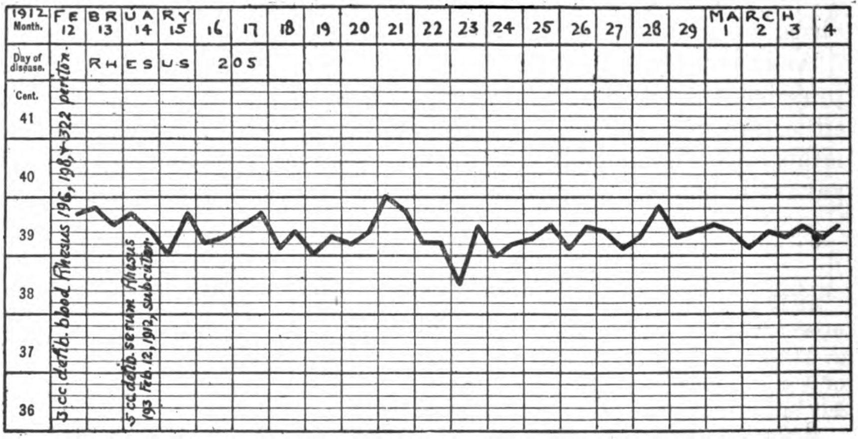

**Chart No. 39.**—Temperature curve of rhesus No. 205, showing preventive action of immune serum given 48 hours after injection of the virus.

*Result:* During a period of observation of 37 days this animal presented no recognizable evidence of reaction (chart No. 39). On March 30 monkey No. 205 was subjected to an immunity test. Twenty-one days later, having in the meantime presented no reaction, he was subjected to a second, on May 9 to a third, and on May 31 to a fourth immunity test without eliciting any recognizable response.

It would, therefore, appear that monkey No. 205 (like No. 215, experiment No. 18) had been saved from infection by the injection of the immune serum 48 hours after the inoculation with virulent blood; the failure to respond to four successive immunity tests would suggest that, although infection had been prevented, an immunity had been induced.

Discussion.—In considering the significance of the results in this and the immediately preceding experiment we do not disregard the possibility that we are dealing with two naturally resistant monkeys. This possibility can not be eliminated. We believe, however, that the probabilities are against such an interpretation in at least one of the experiments. Another suggestion that occurs is that the resistance displayed by these monkeys is due to a passive immunity. This interpretation seems untenable, in view of the results in the first three of this series of experiments.

On the whole it seems most probable that failure of infection in at least one of the two experiments under discussion was due to the action of the immune serum, and that the resistance to the subsequent immunity tests was due not to a passive but rather to an active immunity comparable to the active immunity induced by injections of partially neutralized mixtures of diphtheria toxin and antitoxin.

Summarizing the foregoing experiments we find that they naturally fall into two groups, one comprising four experiments, in which the inoculation with the virus and the injection of the typhus-immune serum were practically simultaneous, the other, comprising two experiments, in which the inoculation with the virus was not followed by the injection of typhus-immune serum until after 48 hours.

In three of the four experiments of the first group the monkeys did not become infected; in the fourth, the monkey developed a well-marked febrile reaction; in the former, the immune sera were obtained between the sixth and the fourteenth days of convalescence; in the latter, the serum was obtained on the thirtieth day.

These results appear to be in harmony with those of Nicolle and Conseil, and of Gaviño and Girard.

In neither of the two experiments comprising the second group did the monkey become infected; in both instances the monkey subsequently resisted repeated immunity tests.

CONCLUSIONS.—From our experiments we may conclude:

1. That it is almost certain (on account of the lack of a uniform susceptibility of the monkey we can not be certain) that immune serum obtained between the fifth and the fourteenth days of convalescence has protective value when injected simultaneously with or within (or, at least, up to) 48 hours after the inoculation of the virus.
2. That the immune serum obtained on the thirtieth day of convalescence had no appreciable protective influence under the conditions of its employment.
3. That when serum and virus are inoculated simultaneously, under the conditions of our experiments, no immunity results.
4. That it is highly probable that when the serum is injected 48 hours after the virus, immunity may, or does, result. This we think is probably of the nature of an active immunity.

## THERAPEUTIC VALUE OF IMMUNE SERUM.

Nicolle and Conseil (September, 1910, and February, 1911), by showing that typhus-immune serum is probably possessed of a preventive action, placed the study of the therapeutic value of such serum on a rational basis. They were also the first to experiment therapeutically with such serum in the monkey, and concluded that it had definite curative power.

Following Nicolle and Conseil came Gaviño and Girard (Nov. 12, 1911), who report the favorable effects from the use of immune serum in one case of typhus.

In a recent communication Nicolle and Conseil (May 25, 1912) report that, encouraged by their first experiments, they made some tests on a somewhat more extended scale, but that their results did not come up to their expectations. Although they worked under the most favorable conditions, using serum obtained between the fifth and eighth day of convalescence and in repeated or in massive doses, in none of the five cases so treated was the duration of the infection appreciably shortened. In these tests they worked with human subjects and used human (filtered) serum.

In the course of our tests of the preventive power of immune serum we also made some trials of its therapeutic value. We treated four monkeys, the subjects of experimental typhus, with monkey-immune serum obtained between the eighth and the eleventh day of convalescence. The sera were all preserved with a little chloroform, and kept at 15° F. for periods up to 46 days before being used. In one of the four animals a single injection was given, in the other three the injections were repeated three or four times. The total amounts administered varied from 20 c. c. to 40 c. c.

We were unable to detect any influence on the course or duration of the fever assignable to the action of the serum, and therefore do not consider it necessary to publish our protocols. Although final conclusions as to the therapeutic value of typhus-immune serum can not be formulated on the basis of so small a material, it is clear nevertheless, and in this we agree with Nicolle and Conseil (May 25, 1912) that such value as it may have is at best slight and at the present time of no practical importance.

## VACCINATION.

The desirability of a method of immunization to typhus without the peril incident to an attack of the disease is obvious. This might be accomplished by inoculations with an attenuated or dead culture of the virus, but unfortunately no way of successfully cultivating the virus has yet, so far as we are aware, been devised. It occurred to us, however, that considerable quantities of the virus might be obtained by simply collecting suitable numbers of infected insects. Accordingly we decided to try to immunize a monkey by injections of increasing quantities of heated body lice that for this purpose were assumed to be cultures of the virus.

### *Experiment No. 20.*

On December 10, 1911, 25 c. c. of a suspension of crushed lice of group No. 9–M, representing 250 body lice, was heated for 30 minutes at 60° C. and afterwards subcutaneously injected into rhesus No. 321.

On December 18, 1911, a suspension of body lice representing 433 of group No. 13–M and 167 of group No. 10–M—that is, 600 in all—were heated at 60° for 30 minutes and subcutaneously injected into rhesus No. 321. Ten days later, namely, December 28, lice No. 16–M, numbering 628, and No. 14–M, numbering 434—that is, 1,072 body lice in all—were crushed and rubbed up in saline solution. This suspension was filtered through glass wool to remove skeletal débris. The filtrate was then heated for 30 minutes at 60° C., after which it was subcutaneously injected into rhesus No. 321.

Lice of group No. 9–M were body lice that had been fed on various cases of typhus during the five days immediately preceding the date of inoculation.

Lice of group No. 10–M were body lice that had been fed on various cases of typhus from December 11 to December 18.

Lice of group No. 13–M were body lice that had been fed on various cases of typhus from December 12 to 17.

Lice of group No. 14–M were body lice that had been fed on various cases of typhus from December 19 to 28.

Lice of group No. 16–M were body lice that had been fed on various cases of typhus from December 21 to 28.

On January 12, 1912, 15 days after the last inoculation, rhesus No. 321 was subjected to an immunity test consisting of an intravenous injection of 3 c. c. of defibrinated blood of rhesus No. 187. To this test rhesus No. 321 responded promptly and sharply. This animal, therefore, was not vaccinated by the injections of heated suspensions of presumably infected body lice.

CONCLUSION.—This single experiment does not permit of definite conclusions. We record it at this time, as it is not likely that we will soon have an opportunity to repeat it.

## PERIOD OF INFECTIVITY OF THE LOUSE.

The work of Nicolle, Comte and Conseil, that of Ricketts and Wilder, and our own having established the fact that typhus is transmissible by the body louse, it naturally suggested itself to inquire into the limitations governing the power of the louse to transmit the disease.

Nicolle and Conseil (January, 1911) were the first to try to determine how soon after an infecting feed the louse became infective. They made one experiment which gave the following results:

A. Of two monkeys subjected to bites of lice that had had their infecting feed from one to four days previously, neither became infected. One of them died of a cachexia.

B. Of two monkeys subjected to bites of lice that had had their infecting feed from five to seven days previously, both developed well-marked reactions.

C. One monkey that was subjected to bites of lice that had had their infecting feed 9 to 12 days previously was entirely negative.

The validity of this experiment, however, is not beyond question inasmuch as the cynomolgus monkey on which the lice used in this experiment were fed in the intervals between feedings on the series may have reinfected these insects.

Nicolle and Conseil note also that, although one of the members of their laboratory personnel was bitten by a single louse and another by three lice, neither became infected; the lice in question had had their infecting feed from one to three days previously. They conclude, therefore, that the louse bite is infective between the fifth and the seventh day after the infecting repast and that it does not appear to be infective either earlier or later.

An interesting experiment performed with the purpose of determining how long lice remain infective after feeding on an infected host is reported by Wilder. Wilder subjected three monkeys in succession to the bites of lice that had previously had three successive feeds on typhus patients. Of these monkeys the first was bitten by the lice during the period of 3 to 6 days from their first and 1 to 3 days from their last infecting feed, or during the period of 1 to 6 days; the second was bitten during the period of 6 to 8 days from their first and 4 to 6 days from their last infecting feed, or during the period of 4 to 8 days; the third animal in the series was bitten during the period of 9 to 11 days from the first and 7 to 9 days from the last infecting feed, or during the period of 7 to 11 days.

In the case of the first two monkeys it was impossible to recognize during the following three weeks either elevation of temperature above normal or other sign of sickness, nor was either of the two protected by lice against later immunity tests, both reacting with fever of moderate severity to subsequent inoculations of typhus blood.

The third monkey developed fever after an incubation period of 17 days, which Wilder interprets as typhus. This animal died 16 days after he was subjected to an immunity test without having manifested any evidence of a reaction. At post mortem acute intestinal and pulmonary tuberculosis was found.

Why the first two monkeys of the series were not infected, Wilder thinks, may be explained by individual variations in susceptibility due to age. "They were both quite young and exceptionally healthy animals. It is conceivable also that the infectivity of the louse increases progressively after his infection due either to the greater proliferation of the organisms, an increase in virulence of the microorganisms while in the louse, or possibly to their migration to the salivary glands."

If we accept Wilder's interpretation of the fever noted by him in the third of the series of monkeys it follows that the bites of these lice were infective during the period of 7 to 11 days after the infecting feed; or, more precisely, inasmuch as the second one of the series of monkeys was bitten by these insects up to and including the eighth day after their first infecting feed, that the bites of these lice were not infective until sometime between the ninth and the eleventh day.

Our own experiments seem to point to the possibility that lice may be infective within four days after the infecting feed.

### *Experiment No. 21.*

On February 12, 1912, some body lice (group 4–N) were obtained and the following day, February 13, were applied to rhesus Nos. 198 and 196, to the former in the morning and to the latter in the afternoon. On the morning of February 14 they were reapplied to rhesus No. 196.

Rhesus No. 198 was in the fifth day and No. 196 in the fifth and sixth days of well-marked typhus reactions, when lice No. 4–N were applied and allowed to feed.

On February 15, about 24 hours after the last feed on rhesus No. 196, 120 of the lice were applied to a fresh monkey, rhesus No. 209. On February 16, 75 were applied, and on February 17 about 40 fed on this same monkey. During the progress of the experiment the lice were kept at 16° to 18° C.

*Result:* Seventeen days after the last exposure to the lice the temperature of rhesus No. 209 began to rise, reaching 40.7° C. in the afternoon of the third day (chart 40). At this time we drew some blood from the heart and after defibrination injected 3.5 cc. of it into the peritoneal cavity of rhesus No. 225. Following the cardiac puncture the temperature of rhesus No. 209 fell and continued to decline; the monkey refused food, remained crouched in its cage, and died five days later. At post mortem the pericardium was distended with blood; there was no other gross lesion present.

The attempt at passage from rhesus No. 209 to No. 225 failed. No. 225 gave no indication of a reaction and later proved susceptible to an immunity test. In spite of this we believe that the elevation of temperature noted in rhesus No. 209 was most probably a beginning typhus reaction, which was therefore induced by the bites of lice during the period of from one to four days after their infecting feed.

### *Experiment No. 22.*

On April 8, 1912, some body lice were obtained at the jail. These, constituting group No. 5–N, were given an infecting feed at 11.15 a. m, April 9 for one hour on rhesus No. 216, then in the fourth day of a marked typhus reaction.

On April 9, 1912, some more lice were obtained. These constituted group No. 6–N and were given an infecting feed at 10.10 a. m. April 10 for one hour on rhesus No. 194, then in the fifth day of a typhus reaction.

CHART NO. 40

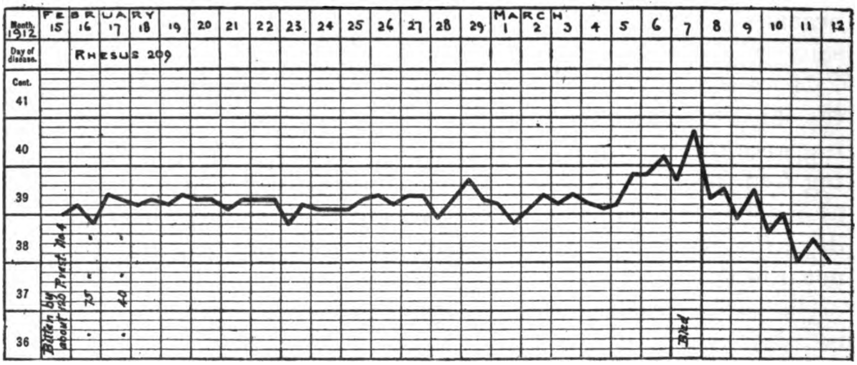

CHART No. 40.—Temperature curve of rhesus No. 209, after being bitten by body lice within four days after their exposure to infection.

Beginning twenty-four hours after their infecting feed lice No. 5–N and No. 6–N were applied on three successive days and allowed to feed on rhesus No. 242, as follows:

April 10, 85 of group No. 5–N.
April 11, 80 of group No. 5–N, and 81 of group No. 6–N.
April 12, 79 of group No. 5–N and 74 of group No. 6–N.
April 13, 67 of group No. 6–N.

They were then applied on three successive days to a second fresh monkey, rhesus No. 245, as follows:

April 13, 61 of group No. 5–N.
April 14, 56 of group No. 5–N and 57 of group No. 6–N,
April 15, 49 of group No. 5–N and 31 of group No. 6–N.
April 16, 17 of group No. 6–N.

During the progress of this experiment the lice were kept at a temperature of 18° to 19.5° C.

*Result:* Neither monkey No. 242 nor monkey No. 245 became infected, nor was either immunized, for both promptly developed well-marked febrile reactions in response to their immunity tests.

The bites of these lice between the first and the sixth days after the infecting feed were therefore ineffective.

### *Experiment No. 23.*

On April 10, 1912, obtained some lice at the jail. These constituted group No. 7–N and were given two infecting feeds, the first at 11 a. m. April 11 for an hour on rhesus No. 194, then in the sixth day of a marked typhus reaction; the second at 12 m. April 12 for an hour on rhesus No. 220, then in the fifth day of a marked typhus reaction.

On April 11, 1912, obtained some more lice which constituted group No. 8–N. These were given a single infecting feed at 12 m. April 12 for an hour on rhesus No. 220 at the same time that group No. 7–N was fed.

Following their infecting feeds these two groups of lice were applied daily, first to rhesus No. 246 then to No. 248, as follows: To No. 246 there were applied on April 13 about 258 of group No. 7–N and 19 of group No. 8–N.

On April 14 about 192 of group No. 7–N and 19 of No. 8–N.

On April 15, 17 of group No. 8–N only.

To rhesus No. 488 there were applied on April 15 about 110 of group No. 7–N.

On April 16, 84 of group No. 7–N and 11 of group No. 8–N.

On April 17, 61 of group No. 7–N and 8 of group No. 8–N.

On April 18, 6 of group No. 8–N only.

*Result:* Rhesus No. 246, bitten by lice within not more than 72 hours after their infecting feed, gave no indications of a reaction during a period of observation of 45 days. Following the immunity test, however, this animal has given evidence of a mild febrile reaction. Rhesus No. 248, bitten by lice during a period of between 2 and 6 days after the infecting feed, failed to present any evidence of a reaction during a period of observation of 38 days, nor has this animal responded in any recognizable way to its immunity test.

It is evident that rhesus No. 246 was neither infected nor immunized. On the other hand, rhesus No. 248, although it gave no evidence of a reaction following the insect bites, appeared to be completely resistant to the (single) immunity test. This is like some of the results of attempts at louse transmission reported by Ricketts and Wilder and by Wilder. The negative result of the immunity test suggests that the bites of the insects, while ineffective so far as febrile reaction is concerned, may have vaccinated the animal.

CONCLUSIONS.—Our experiments are too few in number to permit of a definite statement as to how soon after an infecting feed the bite of the louse becomes infective nor how long this infectivity persists. Nor are we aided materially in formulating definite conclusions on these points by a survey of the literature. Neither Nicolle and Conseil's experiments nor those of Wilder justify the conclusion suggested by the former workers that the bite of the louse is not infective before the fifth day, for one of the experiments herein recorded by us, if it does not prove conclusively, certainly makes it highly probable that the bite of the louse may be infective some time between one and four days after the infecting feed.

As has already been stated, if we accept Wilder's interpretation of the results of one his experiments, it would show that the louse may be infective some time between 9 and 11 days after the infecting feed. While we have no reason to doubt that this may prove to be the case, we do not believe that Wilder's interpretation can be accepted without reservation, inasmuch as the monkey that was the subject of the experiment, the result of which forms the basis of the interpretation in question, was tubercular.

The objection might be raised that our results and those recorded by Nicolle and Conseil and by Wilder are not in harmony one with the other. This is apparently true; but at least one satisfactory explanation of this apparent lack of concordance suggests itself, namely, the individual variation in susceptibility of the monkey. There may be other factors involved and these still remain to be determined.

## CONCERNING THE HEREDITARY TRANSMISSION OF THE VIRUS OF TYPHUS IN THE BODY LOUSE.

The demonstration of the transmissibility of typhus by means of the body louse naturally leads to the question of the transmission of the virus from the infected adult louse to its progeny through the egg. A solution of this question was attempted by Wilder (1911, pp. 87–89).

Wilder reared young lice to maturity on the bodies of typhus patients in order to favor infection of the developing eggs. The eggs laid by these lice were incubated between the sheet and the mattress of a patient in an early stage of convalescence. As the eggs hatched, the young lice were collected and placed on a normal monkey (No. 42); in all, 105 lice—the offspring of infected lice, but themselves never directly infected—were given the opportunity of feeding on this animal. Unfortunately this animal could not be closely observed during the following three or four weeks. A month later, however, he appeared to be in good health and was given an immunity test. On the ninth and the tenth days following this test the monkey showed a slight elevation of temperature which, as Wilder says, may or may not have been an effect of the virus. Wilder adds that "in any case it (the elevation of temperature) is extremely mild, and the animal may be said to have been far less susceptible to an injection of typhus blood than the control or than all normal animals have been found to be." He adds, further, "I appreciate that the result of one experiment does not constitute decisive proof, but the definiteness of the result justifies, in my opinion, the conclusion that monkey 42 owed his immunity to his previous infection by the young lice of group 17, and that hereditary transmission of the infectivity of the louse is established to the extent of reasonable probability."

It is evident that in interpreting the result of this experiment Wilder did not appreciate and did not, consequently, give due consideration to the possibility of a variable susceptibility of the monkey to typhus. It is clear that the slight susceptibility displayed by his monkey 42 may have been, and in the light of our own experience we think it not improbable that it was, due not to the bites of the young lice but to a natural resistance.

We took advantage of an opportunity to repeat Wilder's experiment, as follows:

### *Experiment No. 24.*

A. At 2.30 p. m. December 19, 1911, we applied some eggs (adhering to a portion of a stocking (that had been laid by lice of group No. 10–M to the shaved belly of a normal monkey, rhesus No. 312, and kept them in place by means of a plaster jacket.

Lice of group No. 10–M were body lice that had been applied on four successive days (Dec. 12 to 15) to typhus cases 19–M, 21–M, 22–M, and 23–M, respectively. On December 16, after these four successive feedings on typhus subjects, the lice were put into a stocking and this put on a foot of typhus case No. 22–M, then in the eleventh day of an attack of moderate severity. On December 18, 48 hours after being put on, the stocking with the lice was removed and a considerable number of eggs was found. After removing the lice the stocking with the adhering eggs was put away at air temperature until the next day, December 19, when, as already stated, the eggs were applied to rhesus No. 312 and kept in place by a plaster jacket.

On December 25, six days after applying, the plaster cast was removed and the eggs examined. A considerable number of these had evidently hatched, but only 17 living young lice were found. These were all gorged with blood. They were replaced and the jacket reapplied. Five days later (Dec. 30) the jacket was again removed and the stocking examined, but no recognizable remains of the 17 young lice could be found.

B. January 1, 1912, at between 4.30 and 5.15 p. m., 32 young lice of group 12*a* were applied to rhesus No. 312. Lice of group 12*a*, as will be detailed more fully further on, were hatched between January 30 and February 1 from eggs laid by presumably infected lice of group 12–M. When reapplied to rhesus No. 312, some of them had already fed on the immune on whose foot they had hatched; nevertheless, most of them fed on the monkey.

January 2, 1912, with one more of group 12*a* added to the 32 of yesterday, 33 young lice were applied morning and evening to rhesus No. 312.

At the same time applied 17 young lice of group 11*a*. These had been hatched from eggs laid by presumably infected mothers of group No. 11–M.

January 5, 1912.—The young lice of groups 11*a* and 12*a* have been applied to rhesus No. 312 twice daily since January 2 up to and including this morning.

At 3.15 p. m. 32 of group 12*a* were crushed, rubbed up in saline solution, and subcutaneously injected in this monkey.

C. On January 5, 1912, immediately after the inoculation with crushed lice above mentioned, rhesus No. 312 was subjected to the bites of 37 lice of group 11*b* and to 15 of group 12*b*. These lice had recently been hatched from eggs laid by presumably infected mothers of group 11–M and group 12–M, respectively.

January 6.—The young lice applied yesterday afternoon, reapplied this morning. Applied them again with the addition of 83 freshly hatched, belonging to group 11*b*, and 42 belonging to group 12*b*.

January 7.—In the morning applied all the young lice that were on hand yesterday afternoon. Reapplied them in the afternoon with the addition of 79 freshly hatched belonging to group 11*b* and 34 to group 12*b*.

January 9.—The young lice of groups 11*b* and 12*b* applied to rhesus No. 312 twice yesterday and once to-day.

January 12.—The young lice have been allowed to feed once daily since January 9. To-day, at least five days since they were hatched, all these lice, living and dead, are crushed, rubbed up in saline solution, and injected subcutaneously in rhesus No. 312.

Lice 11*a* and 11*b* were obtained as follows:

A considerable number of young lice were collected and constituted group 11–M. This group was then applied daily between December 12 and 18 to cases of typhus. On December 18 they were put in a stocking, and this put on the foot of a typhus patient (case No. 22–M).

On December 20 the stocking with the lice was shifted to another typhus patient (case No. 25–M). On December 21 the stocking was shifted to a fresh patient (case No. 27–M).

On December 23 the stocking was examined and a few eggs were found to have been laid.

The lice were now transferred to a fresh stocking, and this applied to a typhus patient (case No. 29–M). Six days later—that is, on December 26—this stocking was found to contain a fair number of eggs. The lice were all carefully removed and put into a new stocking; the stocking (No. 21) with the eggs adhering was wrapped in paper and carried close to the body, day and night, by an immune while the fresh stocking with the lice in it was applied to a new patient (case No. 35–M).

On December 29 the stocking containing lice No. 11–M was removed. A considerable number of eggs having been laid, the lice were all carefully segregated and the stocking (No. 22) with the eggs alone was rolled up in paper and kept at air temperature (14° to 22° C.).

December 30.—The stocking containing eggs No. 21 having been carried about on the person of an immune since December 26, it was to-day put on the foot of the same individual. Two days later—that is, on January 1—the stocking was removed and found to contain some young lice (No. 11*a*), a number of which had already fed. With the young lice *in situ* the stocking was wrapped up and kept at air temperature till next morning, January 2, when 17 young lice (11*a*) were isolated and, as already stated, used for feeding on rhesus 312.

January 1.—The eggs adhering to stocking No. 22 having been at air temperature since December 31, were now put on the foot of an immune to hatch. Four days later—that is, January 5—the stocking was examined and 37 young lice (11*b*), most of which had already fed, were found. These were removed and feeding on rhesus No. 312 begun.

The next day—January 6—about 83 more young lice (11*b*) were isolated from this stocking and added to those removed yesterday.

The following day—January 7—79 more young lice (11*b*) were isolated and added to those previously isolated from this stocking, making a total of 199 young lice of group 11*b*, hatched from eggs (stocking No. 22) laid by mothers of group 11–M that had previously been nourished on typhus-fever patients.

Lice 12*a* and 12*b* were young, recently hatched body lice, obtained as follows: First, a large number of eggs were collected and these hatched in stockings on the feet of typhus patients. In this way a considerable number of young lice, which constituted group 12–M, were obtained. These lice, kept in a stocking which was shifted from time to time from one typhus patient to another, began to lay eggs December 23, 1911. The lice were now put into a new stocking and this put on the leg of a typhus patient (case 29–M) and kept in place till December 26, when it was removed and found to contain eggs. The lice were carefully picked from this stocking (No. 23) and put into a new one (No. 24), which was applied to case 35–M. Stocking No. 23, with the adhering eggs, was wrapped in paper and carried close to the body by an immune.

On December 29 stocking No. 24 was removed from the patient's leg, wrapped up in paper, and put away at air temperature 14° to 22°.

On December 30 stocking No. 23, which had been carried wrapped upon the person of an immune since December 26, was to-day put on the foot of the same individual to hatch. Two days later—that is, on January 1—this stocking was removed and was found to contain some young lice, some of which had already fed. These young lice, No. 12*a*, were isolated and feeding begun with them on rhesus No. 312.

On January 1, also, stocking No. 24, that had been at air temperature since December 29, was put on the foot of the same immune, after carefully removing the adult insects and leaving only the eggs. The stocking was examined daily, but no eggs were hatched until January 5, when 15 young lice (No. 12*b*) were found. These had already fed. They were isolated and feeding on rhesus No. 312 begun. The stocking was replaced and next day, January 6, 42 more young lice (12*b*) were isolated and added to those isolated the day before. On January 7 about 24 more young lice (12*b*) were isolated and added to those previously collected. In all, about 91 young lice of this group (12*b*), hatched from eggs laid by mothers which had previously been nourished throughout on typhus-fever blood, were collected and allowed to feed, as already explained, on rhesus No. 312.

Lice groups 12*a* and 12*b*, it will be noted, differ from 11*a* and 11*b* in that the mothers of the former, unlike those of the latter groups, were nourished from birth on typhus blood.

*Result:* Following the above treatment rhesus No. 312 presented no indications of a reaction. On February 23, 42 days after the last inoculation, this monkey was subjected to an immunity test consisting of an intraperitoneal injection of 6 c. c. of mixed defibrinated blood of rhesus Nos. 200, 203, and 204. He responded to this test with a well-marked reaction.

Monkey No. 312 was, therefore, neither infected nor vaccinated by the bites of the progeny of presumably infected mothers.

CONCLUSION.—Our attempt to inoculate a monkey with typhus by means of freshly hatched body lice, the progeny of presumably infected mothers, is frankly negative. Wilder's experiment, when strictly interpreted, must also be considered negative, or, at least, as not satisfactorily positive. It would be premature to conclude from these experiments that the virus of typhus may not pass from the infected mother louse to its progeny through the egg; it is clear, however, that satisfactory evidence that it does still remains to be adduced.

## THE BEDBUG AS A POSSIBLE INTERMEDIARY.

Although all the important features of the epidemiology of typhus are best explained on the basis of louse transmission, and in fact are not explicable in any other way, yet some of the characters might readily be explained on the basis of its transmission by the bedbug.

Recognizing this, Wilder (July, 1911) reports an attempt to infect a monkey by means of the bites of bedbugs that had previously fed on typhus patients. The animal subjected to the bites of these insects showed absolutely no rise in temperature and continued in excellent health. He died from an accident before the immunity test was given. Wilder concludes that this experiment is inconclusive.

We also have attempted to transmit typhus by means of the bedbug and have the following experiments to report:

### *Experiment No. 25.*

Some bedbugs were collected from our guinea-pig pens and these, constituting group No. 1, were given a series of infecting feeds, as follows:

They were given their initial feed in the afternoon of October 25, 1911, by being applied for 20 minutes to rhesus No. 139, then in the third day of a severe typhus reaction. Thereafter they were applied for 20 minutes twice a day to the same monkey until October 31. In the afternoon of October 31 they were given their final infecting feed for 20 minutes on rhesus No. 95, then in the fourth day of a severe typhus.

From November 1 to 7 these insects were applied twice daily to a fresh monkey (rhesus No. 93). At the final feeding 26 survivors were present.

During the progress of the experiment the insects were kept at room temperature in the dark.

*Result:* During a period of 35 days following the last exposure monkey No. 93 gave no indication of a reaction. When subjected to an immunity test he responded promptly and sharply with a severe reaction.

The bugs had therefore neither infected nor immunized this monkey.

### *Experiment No. 26.*

Bedbugs were again collected from our guinea-pig pens and constituted group No. 2. This group was given a series of infecting feeds, as follows:

Beginning October 26, 1911, they were applied for 20 minutes at a time twice daily till October 31 to rhesus No. 140, then at the height of a typhus reaction. They were finally fed in the morning and afternoon of November 1 on rhesus No. 95, then in the fifth day of a severe reaction.

From November 2 to 8—that is, for seven days after the last infecting feed—they were applied to a fresh rhesus (No. 94). At the final feed there were 23 survivors present.

The insects were kept throughout the experiment at room temperature in the dark.

*Result:* The result was precisely the same as in the preceding experiment. Rhesus No. 94 was neither infected nor immunized.

### *Experiment No. 27.*

As in the preceding experiments, we collected some bedbugs, constituting group No. 3, and gave these a series of infecting feeds, as follows:

April 10, 1912, applied to rhesus No. 194, then in the fifth day of a typhus reaction, for 20 minutes.

April 11, again applied to rhesus No. 194 for 20 minutes.

April 12, applied to rhesus No. 220, then in the fifth day of a reaction, for 25 minutes.

April 13, applied to rhesus No. 224, in the seventh day of a reaction, for 15 minutes.

April 14, applied to rhesus No. 220, now in the seventh day, for 20 minutes.

April 15, applied to rhesus No. 223, in the ninth day of a marked typhus reaction, for a final feed of 30 minutes.

April 16, 75 survivors were crushed, rubbed up in saline solution, and then, after allowing the suspension to stand for a minute in a conical glass to permit the coarse particles to settle, all but a little sediment was subcutaneously injected into rhesus No. 249. Throughout the experiment the bugs were kept at 18° to 20° C. in the dark.

*Result:* During a period of observation of 39 days this monkey presented no recognizable evidence of a reaction. On May 31, 1912, this animal was subjected to an immunity test consisting of an intraperitoneal injection of 3 c. c. of virulent blood. During a period of observation of 29 days following this test rhesus No. 249 has failed to give any appreciable indication of a reaction.

It is evident that monkey No. 249 was not infected by the inoculation with the suspension of crushed bugs. Whether the resistance to the immunity test is due to a vaccination can not be said. We are inclined to believe that this is an animal with a high natural resistance to typhus. This experiment is, therefore, inconclusive.

Conclusions.—The failure to infect by means of the bites of bedbugs or by the subcutaneous injection of the crushed insects does not permit of the inference that they were not infected or that they can not transmit the disease. The number of experiments is too few to justify drawing such conclusions. It is to be noted, nevertheless, that such an inference is in harmony with the epidemiological characters of the disease.

## INFECTIVITY OF THE BUCCAL AND PHARYNGEAL SECRETIONS.

To the modern student the epidemiology of typhus speaks loudly against its transmissibility by the "expired air" of the typhus patient. The results of experiments are in harmony with this interpretation of its epidemiology.

In December, 1906, Otero (1908) smeared the throat of a human subject with a gauze swab impregnated with pharyngeal mucous of a patient in the tenth day of illness. Seventeen days later the throat of the same subject was again smeared with a swab impregnated with saliva from a patient in the twelfth day. These inoculations were not followed by any symptoms. That this subject was susceptible is shown by the fact that on a later occasion, following an inoculation with blood, he developed typhus.

As there appears to be no good reason for believing that "droplet" infection plays any part in the transmission of typhus, we have made only a single experiment to test the infectivity of the buccal and pharyngeal secretions, as follows:

### *Experiment No. 28.*

At 1 p. m., December 8, 1911, we gave rhesus No. 310 a subcutaneous injection of 2.5 c. c. of buccal and pharyngeal secretion from patient No. 19–M.

At 4.30 p. m., December 9, rhesus No. 310 was given a second subcutaneous injection of 2.5 c. c. of buccal and pharyngeal secretion from the same patient.

Patient No. 19–M was in the sixth day of a severe attack of typhus, and the blood of this patient obtained on that day was found to be infective for the monkey.

The secretions used for the first injection were obtained two hours before using by having the patient cough and spit into a sterile beaker; those used for the second inoculation were obtained in the same way four hours before injection.

*Result:* Following the foregoing inoculations, rhesus No. 310 gave no indication of a typhus reaction, nor was he found resistant to a subsequent immunity test to which he was subjected 32 days after the second injection of secretions.

CONCLUSIONS.—In itself the negative experimental evidence here cited would hardly justify definite conclusions as to the infectivity of the buccal and pharyngeal secretions of typhus. Viewed, however, in the light of the epidemiology of the disease with which the experimental results are in harmony, we believe it quite justifiable to conclude that the secretions are not infective, and that, therefore, "droplet" infection plays no part in the transmission of the disease.

## SUMMARY AND CONCLUSIONS.

1. A very large proportion (22.5 per cent) of rhesus monkeys possess at least a transient natural immunity, and it seems reasonable to consider that in about 3.5 per cent of monkeys the resistance noted amounts to a permanent natural immunity.
2. A well-marked typhus reaction confers an immunity that may be present after at least two years.
3. Fever is the only definite clinical index of a typhus reaction; when clearly marked it is ordinarily sufficient to justify a diagnosis of typhus.
4. When the fever is slight or its course atypical it can not be construed as a typhus reaction unless the immunity test proves the animal resistant to infection; should the immunity test show that resistance has not been conferred, a diagnosis of previous typhus is not permissible.
5. In about 90 per cent of cases of typhus in the monkey the incubation period varied between 6 and 10 days; the extremes were 5 and 24 days.
6. Although apparently a mild disease, we have had 4 deaths in a total of 103 cases of typhus in the monkey.

7. Repeated inoculations of virulent blood or blood serum, when not followed by a febrile reaction, confer no appreciable resistance.

8. The guinea pig is susceptible to infection with typhus; this animal reacts in essentially the same way to an inoculation with typhus blood as does the monkey. One attack confers immunity from a second.

9. A very small proportion of rabbits may be susceptible to infection with typhus.

10. The immunity conferred by an attack of typhus is specific.

11. Typhus-immune serum obtained between the fifth and the fourteenth day of convalescence has protective value when injected simultaneously with, or within 48 hours after, the inoculation of the virus.

12. Immune serum obtained on the thirtieth day of convalescence probably has no appreciable protective influence.

13. When immune serum and virus are inoculated simultaneously, no immunity results.

14. When the immune serum is injected 48 hours after the virus, an immunity, probably active in nature, may perhaps result.

15. The therapeutic value of immune serum is at best slight, and at the present time of no practical importance.

16. Vaccination with heated crushed-lice suspension was attempted, with negative result.

17. The bite of the body louse may perhaps be infective within four days after the infecting feed.

18. An attempt to inoculate a monkey with typhus by means of freshly hatched body lice, the progeny of presumably infected mothers, was frankly negative. Satisfactory evidence of hereditary transmission of the virus in the louse remains to be adduced.

19. Attempts to transmit typhus by means of the bites and subcutaneous injection of crushed bedbugs were unsuccessful. This result is in harmony with the epidemiological characters of the disease.

20. The buccal and pharyngeal secretions of typhus are not infective, and therefore droplet infection plays no part in the transmission of the disease.

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## HYGIENIC LABORATORY BULLETINS OF THE U. S. PUBLIC HEALTH SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

†No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

†No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

*No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane, by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

*No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

*No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

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